Notes from the Group of Editors

This version of Scientifur, which is the third issue of volume 28, contains the proceedings of the VIII International Scientific Congress in Fur Animal Production, held in 's-Hertogenbosch, The Netherlands, 15 – 18 September 2004.

Please note that the papers in the proceedings have been divided into the following two groups:

RP – reviewed scientific articles and

P – short communications

On behalf of the
Group of Editors

Birthe Damgaard

Erratum to table 5, page 124,

in the article

"Ideal Protein for Mink (Mustela vison) in the Growing and Furring Periods"

Peter Sandbol, T.N. Clausen and C. Hejlesen.

Scientifur, Vol 28, No 3, proceedings,
VIII International Scientific Congress in Fur Animal Production,
's-Hertogenbosch, The Netherlands, 15-18 September 2004,

Due to faulty conversion from g/100 kcal ME to g/MJ, the whole table in the publication shows incorrect values. However this does not change anything else in the publication. The correct figures are as shown below:

Table 5. Estimated content of amino acids in gram/MJ during the growing period, compared to an Ideal Protein (IP) and the present norm.

ME from protein, %	32	28	24	20	16	22					IP	Present Norm
						→ 30	→ 26	→ 22	→ 18	→ 14		
Met incl, MHA*	0.62	0.53	0.46	0.38	0.31	0.57	0.50	0.43	0.34	0.26	0.38	0.38
Met	0.34	0.30	0.25	0.21	0.17	0.31	0.28	0.23	0.19	0.15	0.38	0.38
Cys	0.23	0.20	0.17	0.14	0.11	0.22	0.19	0.16	0.13	0.10	0.14	0.14
Lys	1.03	0.91	0.77	0.65	0.53	0.98	0.83	0.72	0.57	0.46	0.65	0.65
Thr	0.72	0.65	0.55	0.46	0.36	0.67	0.60	0.50	0.41	0.31	0.46	0.46
Trp	0.23	0.20	0.17	0.14	0.11	0.21	0.18	0.15	0.13	0.10	0.14	0.14
His	0.43	0.36	0.31	0.26	0.21	0.38	0.34	0.29	0.23	0.19	0.26	0.36
Phe	0.89	0.79	0.67	0.55	0.46	0.83	0.72	0.62	0.50	0.38	0.53	0.69
Tyr	0.69	0.60	0.53	0.43	0.34	0.65	0.55	0.48	0.38	0.31	0.43	0.43
Leu	1.68	1.46	1.27	1.05	0.83	1.58	1.37	1.15	0.93	0.74	0.95	1.20
Ile	0.77	0.67	0.57	0.48	0.38	0.72	0.62	0.53	0.43	0.34	0.41	0.62
Val	1.03	0.89	0.77	0.65	0.50	0.95	0.83	0.69	0.57	0.46	0.53	0.83
Arg	1.20	1.03	0.89	0.74	0.60	1.13	0.95	0.81	0.67	0.53	0.74	0.74
Gly	1.15	1.01	0.86	0.72	0.57	1.07	0.93	0.79	0.65	0.50		
Ala	1.10	0.95	0.81	0.69	0.55	1.03	0.89	0.74	0.62	0.48		
Ser	0.91	0.79	0.67	0.57	0.46	0.83	0.74	0.62	0.50	0.41		
Asp	1.39	1.20	1.03	0.86	0.69	1.29	1.13	0.95	0.77	0.60		
Glu	2.49	2.18	1.86	1.56	1.25	2.32	2.01	1.70	1.39	1.07		
Pro	1.15	1.01	0.86	0.72	0.57	1.07	0.93	0.79	0.65	0.50		

Table of Content

Welfare and Ethics I			
Author	**Title**	**Number**	**Page**
Ahola	Group housing may impair fur quality in raccoon dogs	I – 4	4
Korhonen	Comparison of hall and shed as housing environments for blue foxes	I – 6	7
Jeppesen	Mink welfare improved by combined implementation of several small initiatives	I – 7	11
Hansen	The anticipatory behaviours of mink expecting a positive or negative reward	I – 8	19
Trapezov	Have fur bearers become domesticated ?	I – 9	24
Plotnikov	Evaluation of comfort of fur-bearing animal keeping by analyzing behaviour	I – 11	33
Trapezov	Conflicts arise between minks of different behavioral types	I – 12	36
Trapezov	Effect of coat color mutation in mink on the adrenal cortex function at pelting time in siberian climate	I – 13	42
Bespyatykh	Young nutria behaviour in runs of different types	I - 14	44

Health II			
Author	**Title**	**Number**	**Page**
Møller	Management of health in mink, *Møller* (keynote)	II – 1	50
Christensen	A preliminary linkage map of the mink (Mustela vison) genome	II – 2	58
Slugin	Not diagnosed stage of aleutian disease.	II – 3	61
Fedoseeva	Characteristics of some morphological and biochemical indices of marmots bred in cages	II – 5	65
Bis-Wencel	A level of some indices of the oxidation state in blood plasma of mink at slaughter period under the definite maintenance and feeding conditions	II – 6	68
Rouvinen-Watt	Mink nursing sickness survey in North America	II – 7	71
Hynes	Body condition and glycemic control in mink females during reproduction and lactation	II – 8	79
Clausen	Wet kits in mink, a review	II – 10	87
Domski	Oral immunization of fur-bearing animals against salmonellosis	II – 14	91

Nutrition III			
Author	**Title**	**Number**	**Page**
Polonen/ Sonderup/ Koenen	A systematic approach to sustainable fur farming, with special reference to feed and feeding **(keynote)**	III - 1	96
Brzozowski	Influence of using enzymatic preparations: α-amylase, β-glucanase and xylanase on fur quality and nutrient digestibility in polar foxes	III - 2	100
Hansen	Different ratio between n-6 and n-3 fatty acids in diets for lactating mink (Mustela vison) dams – effect on milk and kit tissue fatty acid composition.	III - 3	103
Laerke	Physico-chemical properties of different carbohydrate sources in the gut of mink	III - 4	110
Valaja	Effect of lactic acid bacteria and β-glucanase treatments on the nutritive value of barley for growing blue fox	III - 5	116

Nutrition III			
Author	**Title**	**Number**	**Page**
Sandbol	Ideal Protein for Mink (Mustela vison) in the Growing and Furring Period.	III – 6	120
Rouvinen-Watt	Effect of feeding intensity on body condition and glycemic control in mink Mustela vison	III – 7	129
Nielsen	The effect of ad libitum and restricted feeding on growth curves and growth rate curves in mink selection lines	III – 8	136
Leoschke	Sodium BiSulfate As A Mink Feed Preservative	III – 9	140
Ahlstrom	Gas-producing microorganisms in formic acid preserved poultry by-products	III – 10	142
Kulikov	The importance of protein for young mink fed with dry feed	III – 12	147
Kvartnikova	Nourishing qualities of APK concentrate for minks, cubs	III – 13	150
Møller	Individual ad libitum feeding of male + female pairs of mink kits during the growth period increases feed intake, weight gain and feed efficiency	III – 14	154
Nenonen	Effects of intensive fasting and methyl groups of feed on liver metabolism and welfare in breeding blue fox (Alopex lagopus) vixens	III - 15	159
Bis-Wencel	The biochemical parameters in serum of mink fed high energy feedstuff with antioxidants and preservatives supplement.	III – 16	165
Clausen	Correlation between liver fat and dry matter in mink (Mustela vison)	III – 17	168
Damgaard	Effects of feeding strategy on behaviour, physiological parameters and feed residues in mink females	III - 18	170
Glover	Regulation of lipid and glucose metabolism in the mink (Mustela vison) – Sequence analysis and development of molecular probes	III - 19	174
Hejlesen	The effect of protein level on N-balance in adult mink (Mustela vison)	III - 20	182
Skrede	Bacterial protein produced on natural gas as a protein scource in dry diets for the growing-furring blue fox	III - 21	188

Genetics and Reproduction IV			
Author	**Title**	**Number**	**Page**
Hansen	Stochastic simulation of breeding schemes to improve economic genetic merit in mink production	IV – 1	194
Johannessen	Inbreeding in a commercial fur animal breeding programme	IV – 2	201
Peura	Genetics of littersize, age at first insemination and animal size in blue fox (Alopex lagopus)	IV – 3	206
Murphy	Diapause, implantation and placentation in the mink: A critical role for embryonic signaling.	IV - 6	211
Nowakowicz-Debek	The effects of air pollutants on the cortisol and progesterone secretion in polar fox (Alopex lagopus)	IV - 7	218
Sugrobova	The influence of antioxidant emicidin on minks' physiological condition and reproduction	IV - 8	222
Sciesinski	The measurements of the skin electrical conductivity in the acupuncture points affecting reproduction in female polar foxes, Alopex lagopus, during the estrus period	IV - 9	225
Farid	Isolation of microsatellite markers for American mink (Mustela vison)	IV - 10	228
Felska	Liter size, weaning success, and nursing mortality in chinchillas (Chinchilla lanigera) in relation to cage illumination	IV -11	234
Jakubczak	Evaluation of pastel fox breeding results in Poland - body confirmation	IV - 12	238
Jezewska	Genetic variability of choosen conformation traits in chinchilla	IV - 13	244

Genetics and Reproduction IV			
Author	**Title**	**Number**	**Page**
Socha	Genetic and phenotypic parameters of animal size and fur traits in Common Silver Fox (Vulpes vulpes L.)	IV - 14	248
Socha	Genetic parameters of size and fur quality in a mink population (mustela vison sch.)	IV - 15	251
Sulik	Comparison of reproduction management intensity of three genetic lines of female chinchillas (Chinchilla lanigera M.)	IV - 16	254
Szeleszczuk	Morphological changes of spermatozoa in breeding Raccoon dogs semen during cryopreservetion	IV - 17	259
Szeleszczuk	Induction of estrus and ovulation in breeding chinchilla by GnRh analogues	IV - 18	263
Collins	Growth parameters and organ size of American marten (Martes americana) born in captivity	IV - 19	266

Fur Properties V			
Author	**Title**	**Number**	**Page**
Blomstedt	The influence of pelting time on pelt characteristics in blue fox (Alopex lagopus)	V - 2	272
Trapezov	Population genetics and registration of fox pelts in warehouses. (khlebnikov’s travel notebooks revisited in terms of the hardy-weinberg law by borodin)	V - 3	277
Kondo	Hair density and morphology of medulla in Mustelidae	V - 4	283

SCIENTIFUR
ISSN 0105-2403
Vol. 28, No. 3

Proceedings of the VIII International Scientific Congress in Fur Animal Production

I: Welfare and Ethics

Edited by:

Dr. Bert Urlings
Prof. Dr. Berry Spruijt
Dr. Marko Ruis
Ing. Louise Boekhorst

I – 4 RP

Group housing may impair fur quality in raccoon dogs

L. Ahola, S. Hänninen, T. Pyykönen, J. Mononen
Institute of Applied Biotechnology, University of Kuopio
POBox 1627, FIN-70211 Kuopio, Finland
leena.ahola@uku.fi

Abstract

In the wild, the raccoon dog (*Nyctereutes procyonoides*) is a rather social species. Therefore, to meet the species-specific needs of this species also on farms, group housing of farmed raccoon dogs could be considered as an alternative, enriched way of housing these animals. Group housing may, though, affect fur quality e.g. due to aggressiveness or play behaviour between the group mates. In the present study, effects of group housing on production-related parameters were established in farmed raccoon dogs. The animals were housed either as litters (three male and three female siblings in a so-called row-cage system) or as conventional male-female sibling-pairs (in a traditional cage) throughout their growing season. The body mass of the animals, the length of the skins and the number of bite scars in the skins did not differ between the litters and the pairs ($p>0.05$, GLM for repeated measures). Fur quality was worse ($p<0.05$) in the litters than in the pairs. Despite impaired fur quality in the litters, no difference in the price of the skins was emerged between the litters and the pairs ($p>0.05$). In conclusion, group housing of farmed raccoon dogs may, due either to altered physical or to social conditions, impair the fur quality of the animals.

Introduction

According to the recommendations concerning fur animals and their welfare (European Convention, 1999), it is important that the animals have e.g. a stimulating environment appropriate to meet the species-specific needs, including for social species an opportunity to show social investigation and behaviour. In the wild, the raccoon dog (*Nyctereutes procyonoides*) is a rather social species: both parents take care of the young and the cubs may stay their first winter with their mother (Kauhala, 1998). Accordingly, group housing of farmed raccoon dogs could be considered as an alternative, enriched way of housing these animals. Housing fur animals in larger groups may, though, affect the fur quality e.g. due to occasional aggressiveness or play behaviour between the group mates. To clarify the effects of group housing on production-related parameters, farmed raccoon dogs were housed either in groups comprising of three male and three female siblings or in traditional male-female sibling pairs.

Material and methods

The present study was performed during the years 2000 and 2001 at the Research Station of Institute of Applied Biotechnology (University of Kuopio, Finland). Altogether 168 raccoon dog cubs from 28 litters were included in the study during these two years. The raccoon dog cubs were weaned from their mother at the age of approximately eight weeks. Thereafter the litters were divided evenly into two experimental groups that were matched with the cubs' date of birth and litter size. Three male and three female cubs from each litter were transferred into their experimental cages.

One half of the litters (i.e. 36 and 48 cubs in years 2000 and 2001, respectively) was housed throughout their growing season in groups comprising of three male and three female siblings (the sextet group, S). The housing system for the S litters was constructed out of three traditional cages (115 x 105 x 70 cm, L x W x H) that were connected together with openings (30 x 30 cm, W x H) through the walls between the cages (a so-called row-cage system). The other half of the litters was housed as conventional male-female sibling pairs (the pair group, P), i.e. the P litters comprising of three male and three female cubs were divided to live in three separate traditional cages as sibling pairs. Thus, space allocation was 0.6 m^2 per animal in both groups. The matched pairs of litters in the S and P groups were placed into one outdoor fur shed so that in both cage rows of the shed every other three cages were connected together to form a three-cage system (a row-cage system for one sextet) and every other three cages were kept as such (three traditional cages for three male-female sibling pairs).

The raccoon dog cubs were fed, according to the recommendations given by the Finnish Fur

Breeders' Association, on fresh fur animal feed twice a day until late September, thereafter once a day. The daily feed portion per animal was the same for each group. In the S and P group, the feed was delivered onto three trays of the row-cage system and onto the only feeding tray of the cage, respectively. Straw was available for the animals during the whole experiment to meet the animals' nutritional fibre needs. Water was available *ad libitum* until it froze and thereafter twice a day. The health of the raccoon dog cubs was checked daily.
The body mass of the animals was monitored at weaning and at pelting. After pelting, the severity of bite scars was recorded from the leather side of the fleshed skins using a subjective scale from zero (no scars) to seven (plenty of scars). The length of the skins was measured from dry skins from the tip of the nose to the base of the tail. Professional fur graders at the Finnish Fur Sales Ltd (Helsinki, Finland) evaluated the overall quality (density, cover, quality) of the furs using a 10-point scale (1: poorest, 10: best). Also the prices of the skins were obtained from the Finnish Fur Sales Ltd.
Because the cubs within each litter cannot be considered as independent from each other, mean values of measured parameters within each litter, separately for male and female cubs, were used in statistical analyses (Martin & Bateson, 1993). Also the matched-pairs, i.e. one litter from both the S group and the P group that were matched with the cubs' date of birth and litter size, were dependent. Therefore, GLM for repeated measures with both sex and experimental group as within-subject factors was used to analyse the effects of sex and treatment on the measured parameters. Independent-samples T-test was used to analyse differences between the results from years 2000 and 2001.

Results
Within the experimental groups and within sexes (i.e. either male or female cubs in either S or P group), no statistically significant differences emerged in the measured parameters between the two years (2000 and 2001) (for all parameters: $p>0.05$), except in the price of the skins. Therefore, all the results but the price results from these years were pooled together.
During the two years, four and six raccoon dog cubs from S and P groups, respectively, died (or were euthanised) during the experiments. The causes of deaths (or of euthanising) were apparently not due to the housing systems.
There was no difference between the sexes in the body mass at weaning (Table 1). However, at pelting, the males were significantly heavier than the females. Despite this sex difference in the body mass at pelting, there was no difference between the males and the females in the length of the skins. Bite scar score and fur characters as well as the price of the skins in the year 2000 were equal in the males and the females. In the year 2001, the price of the skins was higher for the males than for the females.

Table 1. Body mass (BM, kg) at weaning and at pelting, length (cm) of skins, scar score (8-point scale: 0 = no scars, 7 = plenty of scars), density, cover and quality of furs (10-point scale, 1 = poorest, 10 = best) and price (euros) of skins in raccoon dog cubs housed in sextets or in male-female sibling pairs. G = group (sextets vs. pairs), S = sex (males vs. females). *P*: GLM for repeated measures. *NS*: p>0.05. The data is based on 42 animals in each group (i.e. altogether 168 animals) although statistically the number of subjects is 14 in each group (see Material and methods).

	Males		*Females*		P		
	Sextets N=14	*Pairs N=14*	*Sextets N=14*	*Pairs N=14*	*S*	*G*	*SxG*
BM, weaning	2.5±0.4	2.6±0.4	2.5±0.5	2.6±0.4	*NS*	*NS*	*NS*
BM, pelting	11.1±1.2	11.4±1.4	10.7±1.4	10.8±1.1	*0.009*	*NS*	*NS*
Length of skin	103±3	105±4	103±3	103±3	*NS*	*NS*	*NS*
Scar score	1.5±1.0	1.5±0.9	2.0±1.3	1.7±0.9	*NS*	*NS*	*NS*
Fur characters							
Density	5.7±1.5	7.2±0.7	5.5±1.5	6.9±0.7	*NS*	*0.008*	*NS*
Cover	5.8±0.9	6.3±1.2	5.6±1.2	6.7±0.9	*NS*	*0.034*	*NS*
Quality	6.0±1.9	8.0±0.9	5.9±2.0	7.8±0.8	*NS*	*0.006*	*NS*
Price							
Year 2000	61±8	70±12	60±6	68±9	*NS*	*NS*	*NS*
Year 2001	102±8	113±19	102±14	98±14	*0.045*	*NS*	*NS*

Between the S and P groups, no statistically significant differences were emerged in the body mass of the animals at weaning and at pelting, in the length of the skins, or in the price of the skins (Table 1). The density, cover and quality of furs were significantly lower in the animals housed in sextets than in the animals housed in pairs. Despite impaired fur quality in the sextets, the price of the skins did not differ statistically between the two experimental groups.

Discussion

In the present study, the body mass of the animals and the length of the skins did not differ between the raccoon dog cubs housed in the sextets and in the pairs. This result is in accordance with the earlier results by Korhonen & Harri (1988) who found that group size is the least factor affecting growth performance in farmed raccoon dogs. Thus, it can be concluded that group size does not have any major effects on the growth of raccoon dog cubs.

The number of raccoon dog cubs within the group did not affect the number of bite scars in the skins of the animals. Because the behaviour of the animals was not observed in the present study, it remains unclear whether the bite scars were due to aggressiveness or to play behaviour between the cage mates or were the scars self-inflicted. In the mink, that is considered as a rather solitary species, Hänninen et al. (2002) found that bite scars were more common in cubs housed in litters than in pairs, and concluded that fighting may cause problems in group housed mink. On the other hand, in the silver fox, considered to be a more social species than the mink, the number of bite scars was not affected by group size but more likely by space allocation (Ahola et al., 2002). These results from other farmed fur animals may indicate that bite scars truly reflect the amount of aggression between the animals. Thus, it seems that raccoon dogs, considered as rather social animals, do not fight more in larger groups but are capable of adjusting to variable social environments.

The density, cover and quality of furs were, however, more degraded in the animals that were housed in sextets than in the animals housed in pairs. There may be two reasons for this impairment of fur quality in the S group. First, the raccoon dog cubs housed in sextets were often seen to rest in huddles. This huddling may have abraded the furs of these animals. Second, the animals in the S group were housed in cage systems built up of three traditional cages connected together with rather small openings through the walls between the adjacent cages, and going through these small openings may have caused mechanical wearing of the fur of these animals.

The price of the skins was 4-13 % (years 2001-2000) higher in the pair-housed raccoon dogs than in the sextet-housed raccoon dogs. Although this difference in the price was not statistically significant, the difference is worthwhile to note because the price of skins is still the main factor that affects fur farmers’ willingness to change housing systems and to raise farmed raccoon dogs in merely potentially welfare promoting housing systems.

In conclusion, the present results showed that group housing may, due either to altered physical or to altered social conditions, impair fur quality of farmed raccoon dogs.

References

Ahola L, Mononen J, Pyykönen T, Mohaibes M & Rekilä T. 2002. Effects of group size and space allocation on physiological, behavioural and production-related welfare parameters in farmed silver foxes. Agric Food Sci Finl 11: 185-197

European Convention. 1999. Standing committee of the European Convention for the protection of animals kept for farming purposes. Recommendations concerning fur animals. T-AP(96)19

Hänninen S, Mononen J, Harjunpää S, Ahola L, Pyykönen T, Mohaibes M & Sepponen J. 2002. The effects of family housing on welfare of juvenile farmed mink (*Mustela vison*). In: Koene P, the Scientific Committee of the 36th ISAE Congress (Eds.): Proceedings of the 36th International Congress of the International Society for Applied Ethology. 6-10 August 2002, Egmond aan Zee, the Netherlands. p 87

Kauhala K. 1998. Supikoira. In: Nummi P (Ed): Suomen luonto. Nisäkkäät. Weilin &Göös, Finland, pp.170-175

Korhonen H & Harri M. 1988. Social influences on productive performance in farmed raccoon dogs. Acta Agric Scand 38: 433-439

Martin P & Bateson P. 1993. Measuring behaviour. An introductory guide. 2nd ed. University Press, Cambridge, UK.

I – 6 RP

Comparison of hall and shed as housing environments for blue foxes

Hannu T. Korhonen[1], Teppo Rekilä[1] , Tapani Kivinen[2] and Lauri Jauhiainen[3]
MTT Agrifood Research Finland, [1]Animal Production Research, Kannus, [2]Agricultural Engineering Research, Vihti, [3]Information Services, Jokioinen, Finland
e-mail: hannu.t.korhonen@mtt.fi

Abstract

The study evaluated differences in housing environments between a brand-new fox hall (16 m wide x 75 m long x 7 m high) and a traditional shed. The experiment was carried out during the growing season on two groups of juvenile blue foxes, one housed in a shed and the other in a hall. Each group comprised 50 males and 50 females kept in male-female pairs. The results showed that the temperature was 2-3 °C higher in the hall than in the shed and that relative humidity was 2-4% lower in hall than in the shed. The NH_3 concentration ranged from 0 to 9.5 ppm in the hall but was less than 1 ppm in the shed. The dust concentration ranged from 1 to 2.9 mg/m^3 in the shed and from 0.9 to 3.2 mg/m^3 in the hall. Wind speed was from 0.2 to 0.4 m/s in the shed and from 0.09 to 0.26 m/s in the hall. Average light intensity during Oct-Dec was 4.7 lux in the hall and 5.2 lux in the shed. Sense-based impressions revealed that investigators experienced higher levels of smell and dust in the hall than in the shed but less draught. Substantial differences were not found in body weights, welfare-related variables or fur properties between the groups. Hall conditions seem to be suitable for the commercial raising of juvenile blue foxes.

Introduction

Farmed blue foxes (*Alopex lagopus*) have traditionally been housed in wire-mesh floor cages under shed. Experience of many decades shows that it is possible to produce animals of large body size and good fur quality under such conditions (Korhonen et al. 2000, 2001).However, recent stipulations in environmental and animal welfare legislation, in particular, have put pressures on breeders either to improve the present form of shed housing or to seek alternative housing environments. One potential option would be to raise foxes in fully-covered halls. Such conditions are expected to provide more controlled and stabilized environment, thus, possibly enhancing animal welfare and production performance (Pasanen, 1988; Nydahl & Fors, 1989; Nydahl et al. 1989; Aarstrand & Bøe, 1990). Furthermore, the hall is expected to minimise ground water and air pollution, and make manure handling easy (Kivinen & Rekilä, 2002). Unfortunately, little scientific information is available on the suitability of halls as housing environment for large-scale fox production Therefore, more research on this subject is needed. The purpose of the present study was to fill this gap and to provide measured data on halls and sheds as housing environments for farm-bred blue foxes. Our specific aims were (1) to compare the physical conditions of these two types of housing; and (2) to establish the differences, if any, in production and welfare-related parameters during growing season.

Material and Methods

The study was carried out at the Fur Farming Research Station (of MTT Agrifood Recearch Finland) in Kannus, Finland, (63.54°N, 23.54°E) during July-December 2003. The experimental animals were juvenile blue foxes born in May. Until weaning, they were housed with their mothers and littermates in conventional shed cages 120 cm long x 105 cm wide x 70 cm high. At weaning (age 8 weeks; on July 30), the foxes were divided into two experimental groups: 1) a shed group, with the animals housed in a traditional wire-netting shed; and 2) a hall group, with the animals housed in a hall. The hall was 16 m wide and 75 m long. The height at the ridge was 7 m. Ventilation was natural, gravity based air flow (Kivinen & Rekilä, 2002). Each group comprised 50 males and 50 females housed in male-female pairs. The cage of both groups were 120 cm long x 105 cm wide x 70 cm high. Each cage contained a wire-mesh platform (105 cm long x 25 cm wide) located at about 23 cm from the ceiling and was equipped with birchwood blocks (7 cm long x 5 cm in diameter). Freshly mixed fox feed was supplied twice a day *ad libitum* from commercial feeding machines. The main ingredients of the feed were slaughterhouse offal, fish, fish offal and cereals, in accordance with standard Finnish recommendations. Fresh water was

available *ad libitum* from automatic watering devices.
Ammonia emissions were determined with Dräger ammonia tubes (accuracy ± 1 ppm) at the cage level. The NH_3 indication was based on the colour reaction of ammonia with bromophenol blue and acid. Ammonia changed the colour of the indicating layer from orange to blue, the length of the discoloration indicating the concentration. The indication was evaluated immediately since the colour tended to change somewhat in the course of time. Temperature and relative humidity were measured automatically with tiny tag detectors placed on the ceiling and at cage levels in both the shed and the hall. The dust concentration was measured with Hagner SI Universal (Sweden) equipment at cage level. Wind speed was measured with a Thermo-Anemometer (GGA-26, Finland) at cage level. Light intensity was measured with a photometer at cage level in the shed and the hall and outside the confinements. Sense-based impressions of temperature comfort, smell, draught and dust were evaluated by 10 persons (5 working on the farm, 5 employed in the office building) during the experiment. The evaluation scale was from 1 to 4, where 1= comfortable, 2=fairly comfortable, 3=moderately comfortable, 4=uncomfortable. Manure was collected once a month by Agromatic machine (Bobcat).
Blood samples were collected for determination of the blood picture at pelting (Korhonen et al. 2001). Final body weights were measured on a Vaakakoskinen AD-4326A balance. Adrenals were carefully dissected, cleaned and weighed at autopsy. Mass, quality, cover and purity of colour were evaluated by the Finnish Fur Breeders' Association on a scale from 1 to 10, where 1=poorest and 10=best (Korhonen et al. 2000).
Blood picture, organ weigths and fur properties were evaluated only from males.
Statistical analyses were based on the models accounting for litter as a block effect (Korhonen et al. 2000, 2001).

Results

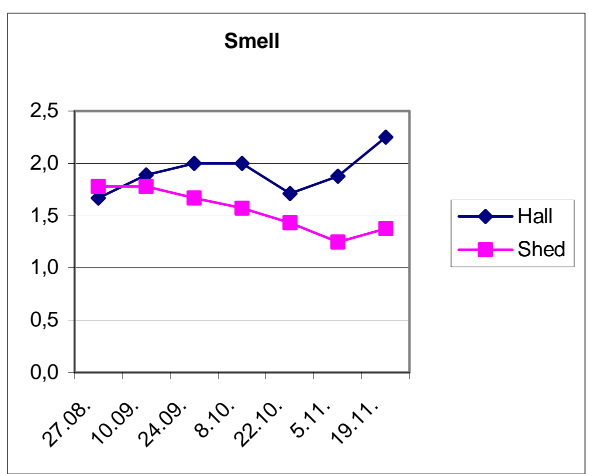
Outside temperatures were typically only a few degrees lower than those in the hall. In August, the air temperature was almost the same in both the hall and the shed. Thereafter, the average temperature was 2-3 ^{o}C higher in the hall than in the shed. The other physical data are summarized in Table 1. Relative humidity was fairly similar in both housing types. No NH_3 concentrations at all were measured in the shed. In the hall, NH_3 concentrations ranged from 0 to 9.5 ppm, the highest concentrations being measured during the carting of manure. Wind speed was higher in the shed than in the hall. Substantial differences were not found in light intensity during Octpber-November. The dust concentration tended to be slightly higher in the hall than in the shed (Table 1).

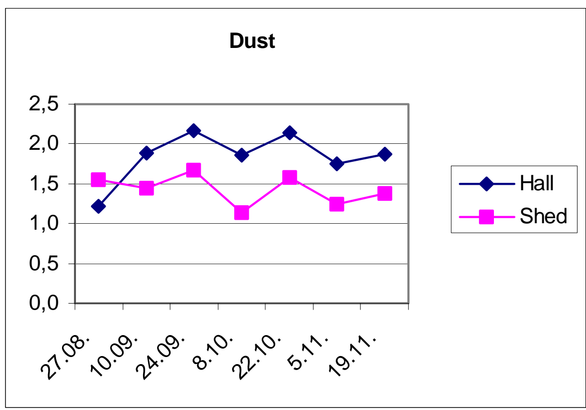
Table 1. Range of physical variables measured under hall and shed conditions. The measurements were made during August-November.

Variable	Hall	Shed
NH_3 (ppm)	0-9.5	0
Dust (mg/m^3)	0.9-3.2	1.0 -2.9
Wind speed (m/s)	0.09-0.26	0.20-0.40
Light intensity (lux)	0-45	0-55
Relative humidity (%)	58-100	57-100

The investigators had a higher sense-based impression of smell and dust in the hall than in the shed (Fig.1). The differences between people working on the farm and in the office building were not significant. The sense-based impression of temperature comfort was fairly similar for both hall and shed. The impression of draught tended to be higher in the shed than in the hall.
Significant differences were not found in final body weights either in males (hall 13.6 kg, shed 13.1 kg, s.e.= 0.33, P=0.22) or in females (hall 11.8 kg, shed 11.3 kg, s.e.=0.22, P=0.08). Nor were there any significant differences in blood picture (haemoglobin: hall 162 g/l, shed 161 g/l, s.e.=1.70, white blood cells 9.0 x 10^9 cells^{-1} vs 8.2 x 10^9 cells^{-1}, s.e.=0.48, red blood cells 8.5 x 10^9 cells^{-1} vs 8.7 x 10^9 cells^{-1}, s.e.= 0.10, haematocrit 51.6% vs 52.6%, s.e.=0.63) or weight of adrenals (hall 317 mg, shed 331 mg, s.e.=19.0, ns). The differences in fur parameters such as quality, mass or cover were not significant. However, purity of colour was significantly poorer in males kept in the hall than in those raised in the shed (6.25 vs 6.94, s.e.=0.17, P=0.006).

Fig. 1. Sense-based impressions of smell and dust during the experiment.





The evalution scale was from 1 to 4 where 1=comfortable, 2=fairly comfortable, 3=moderately comfortable, 4=uncomfortable.

Discussion

When interpreting the present results, we should keep the following points in mind: (1) at the beginning of our growing experiment, in summer, the hall was brand new, i.e. conditions were pristine and clean, and so, we do not yet know how conditions will change in the long run or seasonally.; (2) The animals kept in the hall were all born in a shed, and were not brought to the hall until after weaning. Thus, we have no data on animals born and bred in a hall; (3) comparison of our results with those of previous hall trials is difficult because of the scarcity of data available and because experimental halls differ from each other in structure.

Physical measurements did not reveal any great differences in housing conditions between the hall and the shed. Although NH_3 was detected in the hall, the concentrations measured were lower than the maximum permitted 10 ppm (Ministry of Agriculture and Foresty, 2002). The same holds for dust, i.e, the dust concentrations measured were below the maximum permitted 10 mg/m^3. Sense-based impressions revealed that the investigators experienced higher levels of smell and dust in the hall than in the shed. However, the differences were not striking, and the smell and dust impressions were considered to be within the limit of acceptable sense perception. The NH_3 concentrations reported by Pasanen (1988) ranged from 5 to 10 ppm, i.e. they were very close to those detected here. In another Finnish experiment, Nydahl et al. (1989) measured NH_3 concentrations that were slightly higher than those in our experiment, i.e. from 13 to 25 ppm. They concluded that such concentrations were still well acceptable under hall conditions. In Norway, NH_3 concentrations have been reported to range from 1 to 6 ppm (Bøe & Aarstrand, 1989). Thus, it appears that NH_3 will not be a health problem for either animals or workers in a hall.

Air temperatures inside the hall were measured only during the autumn period. They were either about the same as in the shed or only 2-3 ^{o}C higher. These findings agree rather well with measurements made earlier in Norway (Aarstrad & Bøe, 1990) and Finland (Nydahl et al. 1989). The most interesting periods of the year will be mid-winter and mid-summer, when the temperature differences between hall, shed and ambient air can be expected to be at their highest (Pasanen, 1988). We look forward to evaluating these periods in detail. The present results revealed that wind speed was higher in the shed than in the hall. This may affect temperature comfort in the hall owing to the wind-chill effect, i.e, the combination of higher wind speed and lower temperature will increase the effect of cold. In principle, the relative humidity of the air may also affect the impression and/or impact of temperature. Our measurements revealed that relative humidity in the hall was not substantially different from that in the shed.

According to Pasanen (1988), hall conditions are positive in terms of animal welfare. In the present study, welfare-related parameters demonstrated that the health of our experimental foxes was similar in both housing environments. Thus, hall environment

did not increase the welfare of growing blue foxes. On the other hand, neither was hall housing detrimental to foxes which encourages us to continue this project. It is obvious that further studies will be needed in order to clarify long-term effects on wellbeing.

Our results showed that the production of foxes with large body size and good fur quality is just as possible in halls as in sheds. We conclude that there do not seem to be any obstacles to the commercial raising of growing foxes under hall conditions until pelting. Our next experiments will seek to clarify effects of the hall environment on mating and reproduction. Before we have that information, we cannot draw final conclusions as to the suitability of the present type of hall on commercial fox production as a whole.

Acknowledgements

We thank the staff of the Fur Farming Research Station (MTT, Kannus) for their valuable help in carrying out these experiments. This study was financially supported by the Ministry of Agriculture and Forestry.

References

Aarstrand, K. & Bøe, K. 1990. Klimamålinger i pelsdyrhus. Husdyrforsøksmøtet, Norges landbrukshøgskole, 13-14. February, 1990. pp. 343-347.

Bøe, K. & Aarstrand, K. 1989. Air temperature, air speed and ammonia in buildings for fur animals. IBT-rapport nr. 266. Agric. Univ. of Norway, Dept. of Building Technology in Agriculture, NLH. 19 pp.

Kivinen, T. & Rekilä, T. 2002. New structural solutions for breeding halls in Finland. NJF-seminar no. 347, Vuokatti, Finland 2-4 October, 2002. 4 pp.

Korhonen, H., Niemelä, P., Jauhiainen, L. & Tupasela, T. 2000. Effects of space allowance and earthen floor on welfare-related physiological and behavioural responses in male blue foxes. Physiol. & Behav. 69: 571-580.

Korhonen, J., Jauhiainen, L., Niemelä, P., Harri, M. & Sauna-aho, R. 2001. Physiological and behavioural responses in blue foxes (*Alopex lagopus*): comparisons between space quantity and floor material. Anim. Sci. 72: 375-387.

Ministry of Agriculture and Forestry (Maa- ja metsätalousministeriö). 2002. Sikojen pidolle asetettavat eläinsuojelumääräykset. Asetus nro 14/EEO/2002. 12 pp.

Nydahl, K. & Fors, F. 1989. Farmteknologisk inventering. Finsk Pälstidskrift 61: 200-205.

Nydahl, K.,Fors, F. & Lehtinen, J. 1989. Hallupfödning av pälsdjur – aktuell situation i Finland. NJF-seminarium nr 170, Stockholom 29.9.-1.10. 1980. 9 pp.

Pasanen, S. 1988. Articop Oy:n halliratkaisut, Rääkkylä. Seminaari: Hallikasvatus ja lannanpoistomenetelmät turkistarhauksessa. Fur Center, Vantaa 3.5.1988. 3 pp.

I – 7 RP

Mink welfare improved by combined implementation of several small initiatives

Leif Lau Jeppesen
Biological Institute, University of Copenhagen
Tagensvej 16, 2200 Copenhagen N , Denmark
lljeppesen@bi.ku.dk

Abstract
Several small initiatives, each separately supposed to improve welfare were implemented at one half of a mink farm population: the experimental group (N = 300 females of the colour type wild including males and kits). The other half of the population served as a control group. The initiatives comprised selection for confident behaviour, an empty cage between mated dams, separation of the litter 1-2 weeks after weaning at 8 weeks of age, and furnishing cages with shelves and occupational objects. The initiatives were implemented in the order mentioned from early spring 2003, and the synergistic effect of the initiatives on stereotypies, temperament and pelt damages was measured in kits and in dams during summer and autumn 2003. The results showed that confident behaviour was increased and that frequency of stereotypies and of damages was reduced in the experimental group. It was concluded that welfare was improved by the combined implementation of the initiatives.

Introduction

Current thinking about welfare improvements for mink seems to be somewhat out of focus. The Council of Europe recommended research on swimming and group housing (European Convention, 1999). However, from a biological point of view, access to swimming water and forced cohabitation with conspecifics during group housing are not the first choices of improvements for a species, which is solitary and mainly terrestrial (Dunstone, 1993).

Accordingly, group living and access to swimming water has been shown to impair welfare or to leave it unaffected (Pedersen and Jeppesen, 2001; Hansen and Jeppesen, 2003, 2001).

As mink is an exploring and active opportunistic hunter in the wild, it can be expected that farm housing, in spite of the developed domestication of the species (Kruska, 1996; Kruska and Schreiber, 1999), fails to meet possible needs for being active and exploring. It is also likely, that biologically based fear of humans and neighbouring animals still has to be taken into consideration during future welfare promoting initiatives.

Such initiatives should include further genetic adaptation to farm conditions and proper early stimulation of the animals together with possible changes of the farm environment. The purpose of the present welfare research initiative is to concentrate on minor changes of the conventional cage system since totally alternative environments on the short term are likely to impair welfare in the farm mink that has lived in and adapted to cage systems for more than 100 years (Schackelford, 1984). Several minor changes or initiatives have been shown to improve welfare, but this knowledge is mainly obtained in experimental settings and with smaller group sizes. Recently, Vinke et al. (2002) compared the effect of various degrees of minor changes on five different farms, and they showed that the degree of change was reflected in the level of welfare measured at the farms. The present experiment aims at trying out the combined effect of such minor initiatives by comparing control and experimental groups within one farm population at one large farm.

The implemented initiatives are, in short, (1) to select for confident behaviour, (2) to keep lactating dams and litters in every second cage, leaving an empty cage between them, (3) to wean litters at 8 weeks and keep the kits together (without the dam) for another two weeks, and (4) to provision cages with shelves and occupational object.

Materials and Methods

Animals

The study was initiated by buying a new wild mink stock to the host farm, 600 females and 100 males. The animals were tested for temperament with 10 stick tests (Hansen and Møller, 2001) at the delivery farm, and moved to the host farm in the end of February 2003. The half of each of the sexes that scored confident in most tests was allocated the experimental group. The other half was allocated the control group.

Farm

The animals were housed in a shed with 6 rows of conventional wire mesh cages (30x90x45cm) provided with attached wooden nest boxes covered with straw. The 4 central rows made up the study area with a control section placed in the one end of the rows and an experimental section in the other. In the control section cages were without equipment. In the experimental section each cage was furnished with either a shelf or a tunnel made of wire. The shelf measured 15 x 30 cm and was placed in the back of the cage 10 cm below the roof. The tunnel was a 30 cm long square formed tube measuring 10 cm at each side. One of the flats of the tunnel was connected to the roof in the right side of the cage. Thus, the bottom of the tunnel was in the same distance from the roof as the shelf. Throughout this study tunnels and shelves were used to the same extent, and for easiness they are in the following just referred to as "shelf".

Mating and lactation

The animals were kept individually in adjoining cages during mating (in the beginning of March) and in the control group they were still kept in adjoining cages during lactation and until weaning, which took place when the kits were about 7 weeks old. In the experimental group females were placed in every second cage one week before the earliest expected delivery (in the end of April) and kept with this distance to neighbouring females and litters until weaning, which took place at 8 weeks.

The post-weaning period

After weaning the dams were moved to new cages together with a male or a female kit. Each cage held such a pair of animals. In the experimental section cages were equipped with one occupational object (in addition to the shelf), either a plastic tube, a wire cylinder, or a rope of hemp or sisal. However, the ropes gave rise to problems with the pumps of the farm's mucking out equipment, and they were therefore replaced by sawdust fuel briquettes. The tubes and the cylinders measured 11 x 20-25cm (diameter x length); the briquettes measured 6 x 11 x 15 cm.

The control kits were separated in male-female pairs immediately after weaning. The experimental litters were kept together for another 1 – 2 weeks in one or two cages depending on the litter size. After that, they were separated and the kits kept in pair as in the control group. Experimental pairs were supplied with the same types of occupational objects as the dams from the middle of august

Observations

Qualitative observations were sampled frequently from the onset of the study. Quantitative observations during daytime (09.00-16.00)were performed as follows:

A varying number of lactating dams were scanned 10 times a week while their kits were in their 5th to 7th living week (N = 161, 59 and 198, respectively; almost equally distributed on groups). After weaning, all dams (N = 564) were scanned once a day for 5 days from August 19th to September 15th, and they were tested for temperament once on September 13th. Kit pairs from the control group (N = 512) and from the experimental group (N = 576) were scanned once a day for 5 days between September 1st and September 22nd , and tested for temperament September 12th. During the scanning observations of dams the cages that were adjacent to the feed gangway were observed from the gangway and through the door to the cage. During the scanning observations of kits, the cages that were one row away from the gangway were observed through the back part of the cage.

On the 25th of September, while damages on the summer pelt was still visible, the 512 control pairs and the 576 experimental pairs were examined for damages on ears, neck, back/hips, and tail. Damages were attributed to 3 degrees of severity: insignificant (1), moderate (2), and severe (3). "Severe" implied that larger areas of the pelt were gnawed to the skin. "Insignificant" implied that just single guard hairs were gnawed or damaged.

Behavioural elements

The observed elements are listed in table 1. They are distributed to three categories: positions, behaviours, and social distance. During each scanning observations each of the categories were registered separately, meaning that the three positions amounted to 100 % of the scannings. The same held for the two levels of social distance. Since behaviour could not be observed in the nest, the behavioural categories made up a fraction of the scannings during which the animals were out of nest. For the observations of lactating dams only a limited number of the elements were used (fig. 1); together these made up 100 % of the scannings.

Table 1. Catalogue of behavioural elements distributed to the three categories: position, behaviour and social distance. Elements marked with (d) were registered without regard to category in the first scanning of lactating dams.

Element	Description
Position	
Cage	At least the forepart of the animal visible in the cage, but not on the shelf
Shelf (d)	The animal at the shelf / in the tunnel
Nest (d)	The animal in the nest
Behaviour	
Active (d)	The animal performs other active behaviours than those specified below, e.g. exploration, eating, drinking, defecation, marking, etc.
Object	The animal manipulates the occupational object or passes through
Inactive (d)	The animal is inactive in the cage or at the shelf
Stereotyp (d)	Invariant, five or more times repeated behaviours with no obvious function
In/out (d)	Frequent running in and out of the nest box
Neighbour contact (d)	The dam reacts to the presence or vocalisations of neighbouring dams, mainly by starring at the dam / in the direction of the sound or by starting activity/stereotypy in response to stereotyping neighbours
Play	Social play with cage mate; play fights included when they are performed peacefully, with open mouth display, and without screaming.
Alert	Reaction toward the observer, including flight to the nest-box and staring watching of the observer; to be seen only when observations took place from a distance and through the back part of the cage
Distance	
Alone	The animal has no contact with the cage mate; heads more than 5 cm apart
Together	The animal is in physical or behavioural contact with the cage mate

Temperament
The temperament was measured with a stick test (Hansen and Møller, 2001). The observer inserted a stick through the front door to the cage, waited for 15 seconds, and noted whether the reaction of the animals to the stick/observer was confident (approached the stick curiously, sniffed at it, or manipulated it softly with the teeth), fearful (saw the stick and then actively avoided it), aggressive (attacked the stick violently with claws ant teeth), inattentive (stayed in nest) or uncertain (reacted in a way that could not be assigned to one of the before mentioned categories). It is an as yet unsolved problem for the procedure that animals staying in the nest may do so for two reasons: because they are inattentive or because they are fearful. Since inattentive is usually scored at rather low frequencies it is, however, considered to be a minor problem.

Welfare
Based on Broom and Johnson (1993) the following criteria for good welfare were used: Low levels of stereotypy, of damages, of fear and of overt aggression. Accordingly, high level of confident temperament is also a sign of good welfare.

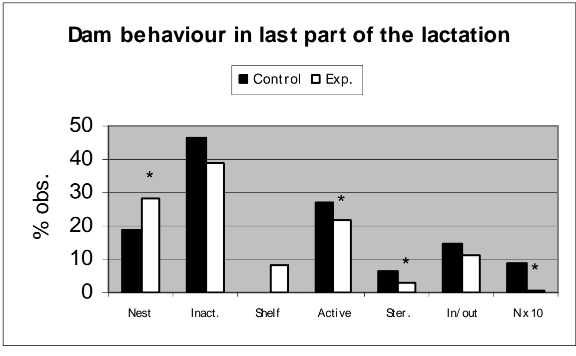
Statistics
Group comparisons: Data on damages were categorical and tested with a two-tailed Chi-Square test. Data on repeated scannings were treated in different ways according to the frequency of occurrence of the elements. For infrequent elements it was noted for each animal whether the element occurred or not in all of the repeated tests, and this individual result was tested with a two-tailed Chi-square test. For frequently occurring elements the proportion of scannings in which the element occurred was calculated for each individual, and this result was tested with a Mann-Whitney U test, since data were not normally distributed.
Time development: The development of the behavioural elements during lactation week 5-7 was tested with a Kruskal-Wallis one-way analysis of variance. All tests were performed according to Siegel and Castellan (1988).

Results

During the first weeks of lactation dams and litters spent most of the time in the nest box and all of the behaviours in the cage increased sharply during week 4 to 5 after delivery. During the quantitative observations in the last part of the lactation, weeks 5 – 7, the experimental dams were more in the nest and they were less active in the cage as compared to control dams (fig. 1). They also performed less stereotyped behaviour, less running in and out of the nest box, and showed less neighbouring contact. They spent about 8 % of the observation on the shelf, mainly by lying inactive. Therefore they were inactive outside the nest to the same extent as the control dams.

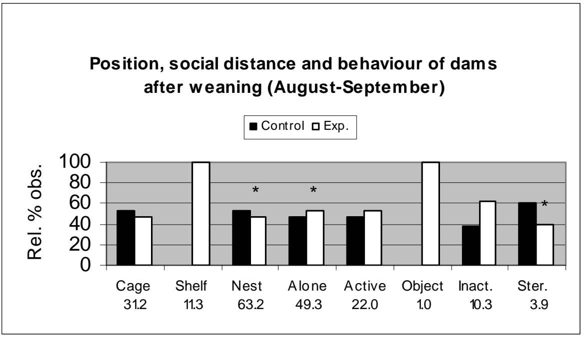
Some of the behaviours developed significantly during the 3 week period. The use of the shelf decreased over the weeks 5, 6 and 7 from 13.2 to 8.5 and 4.8 % of the observations, respectively (P < 0.05). This corresponded well with qualitative observations of the kits' increasing ability to enter the shelf in search of the dam. The frequency of stereotypies increased over the weeks (control dams: 1.5 – 3.8 – 12.2 % of observations, P < 0.001; experimental dams: 0.7 – 0.7 – 4.8 % of observations, P < 0.02). Based on qualitative observation during the 8th week, stereotypies increased further in the experimental group in that week and several kits were bitten and damaged by the dam.

Fig. 1. Average % of observations of selected behavioural elements in the last part of the lactation (weeks 5 to 7 after delivery) in control and experimental dams. * P < 0.05; chi-square test / U-test.



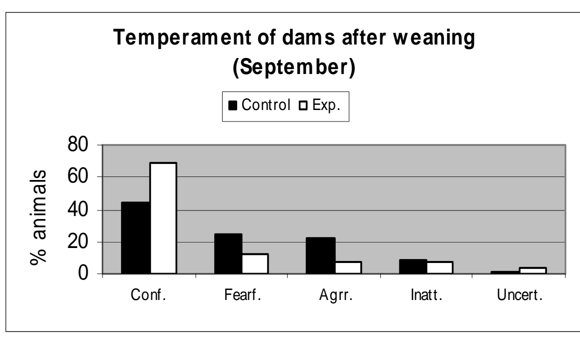
After weaning, the experimental dams used the shelf for about 11 % of the observations (fig. 2). For this reason they were observed less frequent in the cage and significantly less frequent in the nest as compared to control dams. They were also significantly more alone and less stereotyping. They used the occupational object for only 1 % of the time during the quantitative observations. Qualitative observations performed daily during several weeks confirmed this low frequency of use, and gave the impression that plastic tubes and wire cylinders were used more than ropes and briquettes.

Fig. 2. The figures at the bottom show the combined average % of observations in the control and the experimental group, apart from data for shelf and for object that relate to the experimental group only. The columns above show the relative distribution on control and experimental dams. * P < 0.05, Chi-square test / U-test.



The temperament of the experimental dams was significantly different from that of the control dams, the experimental dams being more confident and less fearful and aggressive (fig. 3).

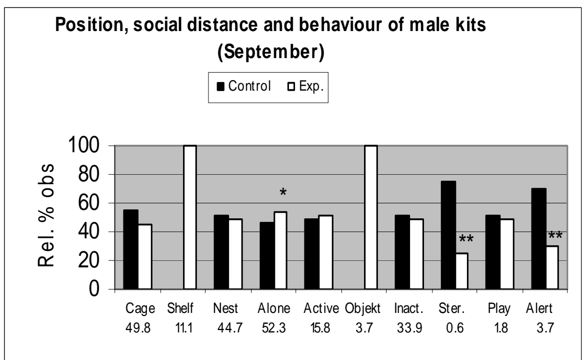
Fig. 3. % control and experimental dams in various temperament categories in September. P < 0.0001, Chi-square test.

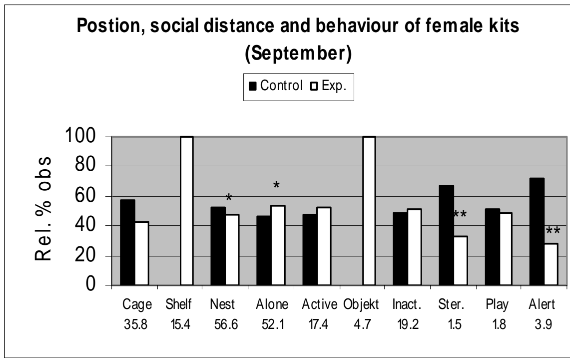


The male and female experimental kits (fig 4) used the shelf 11.1 and 15.4 % of the observations, respectively. As compared to control kits, they were observed correspondingly less in the cage and in the nest (last result significant for females). Both sexes of experimental kits were significantly more alone.

Objects were used 3.7 and 4.7 % of the quantitative observations in male and female kits, respectively. This frequency was much higher during the main activity period in the evening. The plastic tubes and the wire cylinders were used significantly more than the briquettes (P < 0.001, N = 1152, Chi-square test). All objects were manipulated with claws and mouth. Additionally, passage through the tubes and the cylinders was frequently observed. Frequency of stereotypy and of alert watching the observer was lowest in the experimental group.

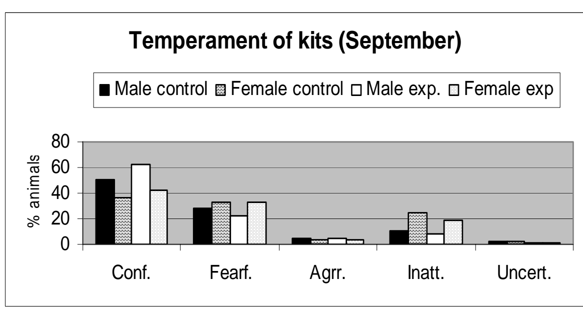
Fig. 4. The figures at the bottom of each panel show the combined average % of observations in the control and the experimental group, apart from data for shelf and for object that relate to the experimental group only. The columns above show the relative distribution on control and experimental dams. Top panel: male kits. Bottom panel: female kits. * P < 0.05, ** P < 0.01, Chi-square test / U-test.





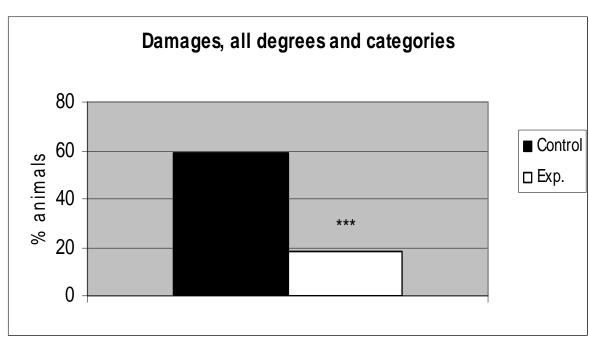
The temperament of control and experimental male kits differed significantly, and the female kits showed the same tendency. Experimental animals were more confident, less fearful, and less in the nest (fig. 5). The differences between the kit groups were smaller than the differences between control and experimental adult females.

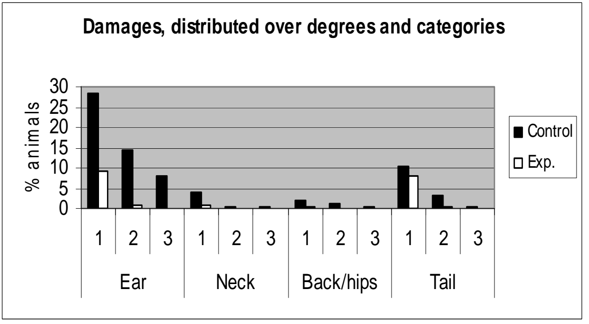
Fig. 5. % control and experimental kits in various temperament categories in September. Males: P < 0.05, females: P < 0.1, Chi-square test.



The number of animals with some degree of damages to the pelt was about 60 % in the control group and about 20 % in the experimental group (fig. 6). Damages to the pelt around the ears and on the tail were most frequent. Some animals had both kinds of damage. Animals with moderate and severe damages amounted to less than 20 % in the control group and less than 1 % in the experimental group.

Fig 6. % of animals with one or more damages (top) and % of damaged animals distributed over degrees and categories (bottom). P < 0.001 for both diagrams, Chi-square test.





Discussion

The present study compares two groups of animals that differ in several ways, since several initiatives were implemented in the experimental group. Therefore, differences between groups may be seen as effects of the combined experimental treatment, and cannot be attributed to any single initiative. For instance, the selection for confidence in the experimental dams may be part of the explanation for any of the observed differences. In the following each initiative is discussed separately in relation to previous literature. Welfare improvement expected from the literature and measured in the present study are attributed, with the above reservation, to the initiative under discussion

The effect of an empty cage between lactating females has been examined before, and so has the effect of various kinds of visual isolation. Recently, Overgaard (2000) showed that reproduction was improved by keeping lactating dams with an empty cage between them. However, this was the case only in primiparous dams of the colour type standard, said to be more stress sensitive than most other colour types. The reproduction of the colour type wild was not influenced by empty cages. Overgaard also noticed that dams located in every second cage were calmer, more in the nest, and not as easily disturbed by neighbouring mink. This was valid for all colour types. Gilbert and Bailey (1967), working mainly with standard mink, found reproduction to be improved by visual isolation of dams from neighbouring dams. Isolation was obtained by placing fibre board partitions between cages. They convincingly explained their result as an effect of reduced social stress (Gilbert and Bailey, 1969). Hoffmeyer and Møller (1986) worked with much larger groups of animals than Gilbert and Bailey and failed to find significant improvements of reproduction as a result of empty or straw filled cages between dams of the colour types standard and pastel. They, too, observed that isolated dams were calmer and less active in the cage.

The results of the present study is in agreement with earlier results, and show further that dams isolated by an empty cage perform less stereotyped behaviour. Together these results show that dams’ welfare is improved by placing them in every second cage, although the effect of the improvement is reflected in the reproduction only in stress sensitive individuals or lines.

During week 5, dams were observed to be on the shelf for 13.2 % of the observations, and this was in sharp contrast to much lower frequency of use in the weeks before. However, as soon as the kits also began to climb to the shelf, dams abandoned this refuge. One likely interpretation is that dams go to the shelf to withdraw from the kits from week 5 onwards, and that they are strained by the kits, when they are deprived this privacy. This might explain the observed increases in stereotypy frequency, as discussed below.

Weaning by removing the dam from the litter took place one week later in the experimental group, and this was supposed to improve welfare in the kits, since it has been shown that individuals that are weaned late show less abnormal behaviour later in life (Mason, 1994; Jeppesen et al., 2000). Whether this is the case also in this study, remains to be examined. However, the pre-weaning welfare seemed to be impaired by the later weaning in the experimental group, both for dams and kits, since several kits were bitten and damaged by the dams, and since frequency of stereotypies in the dams increased sharply in the weeks before weaning. The initiative on later weaning certainly needs further scientific examination. The dams’ damaging and stereotyped behaviour may be caused by the restricted area available for the dam and the litter in conventional cages. If this is right, both pre and post weaning welfare should benefit from later weaning in alternative housing systems allowing dam and litter to share e.g. three cages (e.g. Vinke 2002, Pedersen et al., 2004). However, keeping dams and litter together until pelting time impairs welfare (Pedersen and Jeppesen, 2001).

Selection of mink for confident behaviour towards humans with a stick test has been shown to result in mink that experience less fear in many situations, whether it is in relation to humans or other animals or novel situations (Malmkvist and Hansen, 2002). The stick test has been shown to be applicable at the farm level to select for confident animals (Hansen and Møller, 2001). The experimental kits of the present study were as expected less fearful than control kits. It was shown in the stick test and by the lower frequency of alert behaviour in the scanning observations. An overall reduced level of fearfulness is in itself an improvement of welfare, and the demonstrated effect of selecting for confidence confirm that this initiative is an effective means of improving welfare in praxis.

Conventionally, mink are provided with straw on their nests for most of the year. They use it for nest building, and are occupied by pulling it down, carrying it around in the cage, loosing it, and pulling in new pieces. Maybe therefore, studies on

alternative occupational objects have not received much attention in mink. Jeppesen and Falkenberg (1990) tested the effect of two plastic balls, and although activity with the balls ceased within on month, general activity and curiosity was still increased after that period in mink kits that lived with plastic balls. Vinke et al. (2002, describing a new Dutch housing system) report on use of plastic tubes as occupational objects. Such tubes were also found to elicit the most lasting activity in pilot examinations previous to the present study, and therefore included in the final testing. In the kits, the wire cylinder and the plastic tube were used to the same extent, about 4 % of the time after about one month's use. This is more than previously observed with other objects, and it might have influenced the behaviour and the welfare of the kits, as observed in the behavioural tests and the damage registration. These objects seem to be important enrichments for kits. In the adult animals they are used less frequent.

Both age groups used the shelves in 10-15 % of the observations during the autumn, and they are therefore a much used enrichment of the cage. Although a shelf has room for two or more animals it was mainly occupied by just one individual. This is most likely the main reason that experimental animals were observed to be more alone. Whether mink need to be more alone and therefore benefit from fulfilling such a need is not known. Shelves are part of a new Dutch housings system (Vinke et al., 2002).

The frequency of damages was very high in the present study. The main reason for this may be that there was no selection against this trait, which is genetically based (Nielsen, 1996) and usually selected against at farms. The new breeding animals for the study were bought at farms that kept the least damaged animals for their own use, and the only selection performed during the study was in favour of confident animals. The high damage frequency made it possible to see a marked reducing effect of the experimental conditions on the damages. The reasons for this difference may be any of the implemented initiatives. However, important contributions may come from the shelf and the occupational objects. They were used a lot in the period leading up to the damage registration. The calmer raising conditions in the experimental group could also play a role. The low level of damages in the experimental group underline that welfare is improved by the implemented initatives.

The behavioural observations indicated that experimental animals shoved less stereotyped behaviour than control animals. They were also less fearful based on results from the stick tests and from the scannings, in which they showed less alert watching at the observer. In conclusion, these results suggest that experimental animals experience better welfare than control animals as a result of the combined implementation of the minor initiatives that were tried out in the present study. The only exception was that experimental dams and kits were more stressed due to later weaning. As mentioned, Vinke et al. (2002) also tried out several small changes and showed that the more changes that were implemented on a farm the lower were the stereotypy frequency. A part of their study was to provide larger free area for the dam and litter by allowing them to roam through several joined standard cage until weaning at 11 weeks. This may be a solution for improving the weaning situation. However, because of the experimental design a causal relationship was not implied in their study.

Many of the more extensive initiatives that have been proposed and tried out in the recent years, e.g. introduction of water for swimming and group housing for extended periods (e.g. Hansen and Jeppesen, 2001; Pedersen and Jeppesen, 2001) have failed to show comparable positive effects on welfare or in fact impaired welfare due to the introduction of new problem that the animals are not adapted to cope with. Therefore, the general advice to be taken from the present study is that improving welfare of mink in the short term is best accomplished by smaller improvements of the current management practices and housing conditions for which the mink are adapted. More excessive initiative may well jeopardise the welfare by threatening the adaptive abilities of the species.

References

Broom, D.M. and Johnson, K.G. 1993. Stress and Animal Welfare. Chapman and Hall, London, UK.

Dunstone, N. 1993. The Mink. T & A.D. Poyser, London, UK.

European Convention for the protection of animals kept for farming purposes, Strasbourg, 1976, ETS 87. Recommendation concerning fur animals, 1999.

Gilbert, F.F. and Bailey, E.D. 1969. Visual isolation and stress in female ranch mink particularly during the reproductive season. Canadian Journal of Zoology, 47: 209-212.

Gilbert, F.F. and Bailey, E.D. 1967. The effect of visual isolation on reproduction in female ranch mink. J. Mammal., 48: 113-118.

Hansen, C.P.B. and Jeppesen, L.L. 2003. The influence of temperature on the activity and water use of farmed mink (Mustela vison). Animal Science, 76: 111-118

Hansen, C.P.B. and Jeppesen, L.L. 2001. The swimming activity of farm mink (Mustela vison) and its relation to stereotypies. Acta Agriculturæ Scandinavica, Sect A, 51: 71-76

Hansen, S.W. and Møller, S.H. 2001. The application of a temperament test to on-farm selection of mink. Acta Agriculturae Scandinavica, Animal Science, suppl. 30: 93-98.

Hoffmeyer, I and Møller, S 1987. Afskærmning af minktæver i reproduktionsperioden (Visual isolation of mink dams in the reproductive period). Dansk Pelsdyravlerforening. Faglig Årsberetning 1986, 189-208.

Jeppesen, L.L., Heller, K.E. and Dalsgaard, T. 2000. Effects of early weaning and housing conditions on the development of stereotypies in farmed mink. Appl. Anim. Behav. Sci., 67: 127-135.

Jeppesen, L.L. and Falkenberg, H. 1990. Effects og play balls on peltbiting, behaviour and level of stress in ranch mink. Scientifur, 14: 179-186.

Kruska, D. 1996. The effect of domestication on brain size and composition in the mink (Mustela vison). Journal of Zoology, London, 239:645-661

Kruska, D. and Schreiber, A. 1999. Comparative morphometrical and biochemical-genetic investigations in wild and ranch mink (Mustela vison. Carnivors, Mammalia). Acta Theriologica, 44(4): 377-392.

Malmkvist, J and Hansen, S.W. 2002. Generalisation of fear in farm mink, Mustela vison, genetically selected for behaviour towards humans. Animal Behaviour, 64: 487-501.

Mason, G. 1994. Tail biting in mink (Mustela vison is influenced by age at removal from the mother. Animal Welfare, 3: 305-311

Nielsen, U.L. 1996. Genotype – environment interactions in relation to selection for and against pelt chip in mink. Animal Production Review, Applied Science Report 27: 167-172.

Overgaard, L. 2000. Effekt af tomt bur mellem minktæver i reproduktionsperioden (Effect of an empty cage between female ranch mink in reproduction period). Danish Fur Breeders Research Center. Annual Report 1999: 37-42.

Pedersen,V. & Jeppesen, L.L. 2001. Effects of family housing on behaviour, plasma cortisol levels and production in adult female mink (Mustela vison). Acta Agriculturæ scandinavica, Sect A, 51: 77-88.

Pedersen,V., Jeppesen, L.L. and Jeppesen, N. 2004. Effects of group housing systems on behaviour and production performance in farmed juvenile mink (Mustela vison). Applied Animal Behaviour Science, accepted

Shackelford, R.M. 1984. American Mink. In: Mason, I.L. (ed.). Evolotuin of domesticated animals. Longmann, England, pp 229-234.

Siegel, S. and Castellan, N.J 1988. Nonparametric statistics for the behavioural sciences. McGraw-Hill, Singapore, pp. 399.

Vinke, C.M., Eenkhoorn, N. C., Netto, W.J., Fermont, P.C.J. and Spruijt, B.M. 2002. Stereotypic behaviour and tail biting in farmed mink (Mustela vison) in a new housing system. Animal welfare, 11(2): 231-245.

I – 8 RP

The anticipatory behaviours of mink expecting a positive or negative reward

Steffen W. Hansen[1] & Leif L. Jeppesen[2]
[1] Department of Animal Health and Welfare, Research Centre Foulum, P.O.Box 50, DK-8830, Tjele. E-mail: Steffenw.hansen@agrsci.dk
[2] Department of Animal Behaviour, Zoological Institute, University of Copenhagen, Tagensvej 16, DK-2200 Copenhagen N, Denmark

Abstract
The anticipatory behaviours of high and low stereotyping female mink were observed in relation to either a positive (a tit-bit) or a negative reward (capture in a mink trap). The results demonstrated that mink are sensitive to rewards and have the capacity of distinguishing between positive and negative rewards. The anticipatory responses to a positive reward increased several activities out in the cage. The primary anticipatory response to a negative reward was to stay in the nest box or in the entrance of the nest box. However, the anticipatory responses were influenced by the test situation and the feeding time.

Introduction

It has been argued that welfare is the balance between positive and negative experiences or affective states, and that observing behaviours during anticipation of reward in a Pavlovian conditioning paradigm is an easy and useful tool to assess the state of this balancing system (Spruijt et al., 2001). Behaviour during anticipation of an oncoming positive or negative reward may therefore be used as a valuable tool to assess welfare.
Another tool to assess welfare is the occurrence of stereotypies, which is often considered amongst the most important indicators of long-term animal welfare problems (Broom, 1993). Stereotypies are associated with past or present suboptimal aspects of the environment (Mason, 1991), and therefore they have been used as welfare indicators. However, some studies indicate that stereotypies in farm mink should not unconditionally be regarded as indicators of poor welfare (Wiepkema, 1987; Mittelman et al., 1991, Jeppesen et al., 2004).
In the present study, the anticipatory behaviours of high and low stereotyping female mink were studied when they were expecting either a positive reward (a tit-bit) or a negative reward (catching in a mink trap). The purpose of the study was to identify useful behavioural elements during an anticipation test for the assessment of welfare of farmed mink and to validate the effect of positive and negative rewards on the performance of stereotypies.

Materials and Methods

In this experiment, 48 adult female mink were used. As a criterion for selection of the 48 mink, half of the mink should have performed stereotypies in more than 15 of 54 scans (High-st mink) and the other half should never have been observed performing stereotypies (Low-st mink). The behaviour of the selected 48 female mink was tested (day 0) before the experiment in order to obtain basic knowledge with respect to the behavioural elements 'use of nest box', 'stereotypies', and 'general activity'. All the mink were kept solitary in commercial mink cages with free access to drinking water and a nest box. All of the cages contained solitary mink, however, test mink were situated 2 meters apart. The mink were fed *ad libitum* by a machine at 11 o'clock with feed from a commercial feed kitchen (four out of six mink had feed left over the next day and the left-overs were distributed to the ones without left-overs).
To investigate anticipatory behaviour, a classical Pavlovian conditioning set-up was used, where the unconditioned stimulus (US) was given immediately after the conditioning stimulus (CS) on each trail. This procedure was used for the first 10 days of training. Then another procedure was used for the next 17 days, where the US was not presented until 1 min after the CS had ended. In total, the mink were trained on 27 successive days in the period from 8.30 a.m. to 10 a.m. During the 1 min CS-US interval the anticipatory behaviour of the mink was observed on days 11, 25, 26 and 27.
The CS used in the present studies consisted of either a high or a low tone. The high tone was followed and paired with the US positive reward (a tit-bit) and the low tone was followed and paired with the US negative reward (catching in a mink trap).

Immediately after the CS the mink was offered a tit-bit on a spoon (canned cat food) through the front wire netting of the cage or the cage-door was opened and the mink cached in a mink trap. The trapped mink was taken out of the cage and then the mink was released into the cage again.
Half of the High-st mink and half of the Low-st mink, hereafter called group-PH and group-PL, P indicating the positive reward and H and L indicating a high and low level of stereotypies, respectively, received the positive reward. The other half of the High-st mink and Low-st mink, hereafter called group-NH and group-NL, received the negative reward.
The physical distance between the mink allowed the succeeding mink to hear the CS tone when the preceding mink was trained. Therefore, on days 25, 26 and 27 the behaviour of the succeeding mink was observed during 1 min before the normal testing procedure. This extra test of the succeeding mink was performed simultaneously with the normal testing procedure. These extra tests allowed a comparison between the behaviour during the ongoing test and the behaviour before the test situation, when no person was present in front of the mink, but the mink was aroused by the CS-tone from the previously tested mink.
On the last day of testing (day 27), all the mink were tested five times, two times before feeding (in the period from 8.30 a.m. to 10.30 a.m.) and three times after feeding in the period from noon to 15.00 p.m. This procedure was chosen to see if the behaviour of the mink was affected by the time of feeding.
Two persons performed all the training of the mink. The person who performed training with the positive reward wore orange clothes and the person who performed training with the negative reward wore grey clothes.
The duration of the behavioural elements of each mink was observed during a 1 min continuous sampling on days 0, 11, 25, 26 and 27. The behavioural elements observed are shown in table 1. The combination of the elements of the full repertoire was used on days 1 and 11. The full repertoire was used on days 25 to 27.

Statistics
Data were processed using the Statistical Analysis Systems Institute (1996) program. Anticipatory behaviour was calculated as the percentage of the total observation time. The mean of the behavioural parameters on days 25 and 26, and in the first test on day 27 was calculated for each individual during and before the test situation, and used in the statistic. In the same way the mean of the behavioural parameters on day 27 was calculated per individual before and after feeding. Because the behavioural data violated the assumptions of parametric statistical tests, distribution-free methods were used. Wilcoxon matched pairs test was used between independent groups, and Wilcoxon signed rank t-test between trials within the groups.

Table 1. Catalogue of behavioural elements.

§ Nest box	The mink is totally withdrawn to the nest box.
§ Nest entrance	The mink is lying in the nest box with the head and the forepart outside the box
Stereotypy	Regularly repeated and morphological identical movements without any obvious function (e.g. Bildsøe et al. 1991)
* Normal locomotion	Normal walking around in the cage
* Scratching	Scratching and biting the cage door,
* Standing	Standing in front of the cage door looking at the observer
* in/out	Running in and out of the nest entrance
Other activities	Drinking, eating, defecating, lying, freezing etc. These made up less than 1% of the observations and are not presented

§ on day 11 combined to: Nest
** On day 11 combined to: General Activity*

Results

Behaviour before treatment

Before the start of the treatment on 24 October (day 0) the High-st mink spent less time in the nest box than did the Low-st mink (10.9% vs. 60.2%, $p < 0.001$). There was no significant difference in general activity (High-st mink: 45.5% vs. Low-st Mink: 39.8%). The low level of time spent in the nest box of the High-st mink corresponded to 43.6% stereotypic behaviour out in the cage. The Low-st mink did not perform stereotypic behaviour.

There was no significant difference between the two groups of High-st mink that subsequently received a negative or a positive reward. However, as regards the time spent in the nest box, a difference was found between the two groups of Low- st mink that later received a positive or a negative reward. The Low-st mink that were subsequently given a negative reward (NL) spent more time in the nest box on day 0 (78.0%) and less time out in the cage (22%) ($p<0.05$) than Low-st mink (43.8% and 56.2%, respectively) that subsequently received a positive reward (PL) ($p<0.05$).

Effect of 10 days of treatment

After ten days of training, the positive reward given on day 11 had the effect of group-PH and group-PL spending less time in the nest box (8.1% and 19.4%, respectively) than group-NH and group-NL that received a negative reward ($p<0.001$) (59.2% and 78.3%, respectively). Group-PH performed stereotypic behaviour in 14.4% of the time, whereas group-NH did not perform stereotypic behaviour at all. Group-PL increased the general activity to 80.5% compared to 21.7% in group-NL ($p<0.001$).

Group-PL spent more time in the nest box (19.5%) than group-PH (8.1%) ($p<0.05$), but in contrast to PL, group-PH performed stereotypic behaviour. No significant difference in the behaviour of group-NH and group-NL was found.

Compared to the level on day 0, group-NH increased the time spent in the nest box from 9.8% to 55.5% ($p<0.05$) and reduced the stereotypic activity to 0% ($p<0.01$). As right from the beginning of the experiment the mink in group-NL spent much time in the nest box, the negative reward did not result in a significant increase.

Compared to the level on day 0, group-PH reduced the time spent performing stereotypic behaviour from 45.3% to 14.7% ($p<0.05$), and increased the general activity from 42.8 to 77.5% ($p<0.01$). The mink in group-PL reduced their time spent in the nest box from 43.8% to 19.4% ($p<0.01$), while they increased their general activity from 56.2% to 80.6% ($p<0.01$).

Effect of 25-27 days of treatment

At the test on days 25-27, the previously demonstrated difference in the use of the nest box between mink receiving a positive or negative reward remained significant.

The mink in group-NH ($p<0.05$) and group-NL ($p<0.01$) spent more time in the nest box and in the nest entrance ($p<0.01$) than the mink receiving positive rewards (group-PH and group-PL).

The mink in group-PL spent more time in normal locomotion ($p<0.01$), they spent more time standing at the cage-door ($p<0.001$), they scratched the cage-door ($p<0.05$), and they spent more time moving in/out of the nest box ($p<0.001$) than the mink in group-NL.

The mink in group-PH performed stereotypic behaviour, but not significantly more than the mink in group-NH. The mink in group-PH as well as in group-PL spent more time moving in/out of the nest box than the mink in group-NH and group-NL ($p<0.001$). In general, the High-st mink performed more normal locomotion than the Low-st mink ($p<0.01$).

Effect of observer

No significant effect of the presence of the observer was found on the time spent in the nest box. However, during the test, when the observer was standing in front of the cage, the mink in group-NL spent more time in the nest entrance than when the observer was not present ($p<0.05$). The mink in group-NH performed less stereotypic behaviour when the observer was present than when the observer was not present ($p<0.05$), whereas in group-PH the presence of the observer did not imply a significant reduction in stereotypic behaviour. Without the observer, the mink in group-PH as well as in group-NH spent 30% of their time performing stereotypic behaviour. In the presence of the observer, the mink in group-PH spent 13.2% of their time performing stereotypies, and in group-NH the mink only spent 2.4% of their time performing stereotypic behaviour.

In the presence of the observer, the mink in group-PH spent less time performing normal locomotion ($p < 0.001$), spent more time standing ($p<0.05$), and spent more time moving in/out of the nest box ($p<0.01$). Scratching the cage-door was only observed during the presence of the observer, and only in mink given a positive reward. However, it

was not possible to demonstrate a significant increase in the time spent performing such activities ($p=0.06$ for low-st mink).

Effect of feeding time

The mink were more active before feeding than after feeding. Although all the groups spent more time in the nest boxes after feeding ($p<0.05$), the difference between the mink given a positive reward and the mink given a negative reward remained significant.
The mink in group-PH as well as in group-PL spent more time scratching the cage-door ($p<0.05$) and they spent more time moving in/out of the nest box ($p<0.05$) before feeding than after feeding, however, the mink in group-PH continued to spent more time moving in/ out of the nest box than group-NH ($p<0.05$). The mink in group-PH and group-PL spent more time performing normal locomotion ($p<0.01$) and more time standing ($p<0.01$) than group-NH and group-NL. However, as regards the time spent in the nest entrance, performing stereotypic behaviour, or scratching the cage-door, no differences were found between the mink given a positive reward and the mink given a negative reward. Within each group of treatment no significant differences were found after feeding between High-st mink and Low-st mink. Before feeding, group-PH spent more time performing stereotypies than group-PL ($p<0.05$), and they also spent more time performing normal locomotion ($p<0.05$) and less time in the nest box ($p<0.05$) than did the mink in group-PL.

Discussion

In general, the results demonstrated that mink are sensitive to rewards and have the capacity of distinguishing between positive and negative rewards. The impression was that expectation of a positive reward increased several activities in the cage while expectation of a negative reward increased time in the nest or in the nest entrance. This is in accordance with studies on rats (van den Bos, et al., 2003) and foxes (Moe et al., 2003), and suggests that the methods to access the value of supposed enrichments or the level of experienced welfare (Spruijt et al., 2001) can be developed on the basis of this behavioural response. Stereotypies were seen during the anticipation of both positive and negative rewards, but only in High-st mink. In the present study, the stereotypies during anticipation of a reward decreased on days 11 and 25-27 compared to the level on day 0, and the decrease was more pronounced in group-NH than in group-PH. However this decrease seems to be related to the presence of the observer in the test situation. When the observer was standing in front of the cage, the fear of the negative reward increased the stay in the nest entrance, and thus decreased the stereotypies in group-NH and the normal locomotion in group-NL. When expecting a positive reward, the mink directed their response towards the observer by standing in front of the cage door, scratching the cage door, or by running in/out of the nest box, and thus they also reduced their stereotypies and normal locomotion, even though the reduction in stereotypies in group-PH was not significant. When the observer was not present and the mink, presumably, were aroused by the CS-tone, the levels of stereotypies in group-NH and group-PH were almost at the same levels as before the treatments, and new stereotypies were not developed in group-NL and group-PL. This shows that the performance of stereotypies was affected in the same way by welfare inducing and welfare reducing influences, and that the levels of stereotypy were mainly related to the presence or absence of the observer, acting as a proper stimulus to direct the normal behaviour. This confirms that stereotypies are not always a valid measure of poor welfare (Mason & Latham, 2004; Jeppesen et al., 2004). In a resent study (Vinke et al., 2004) it was not possibly either to find any relationships between anticipatory activity (behavioural transitions) and stereotypies.
The effect of treatments (in nest box, normal locomotion, standing) was not affected by the feeding time. However after feeding, the mink spent more time in the nest box. Within treatment on day 27, no behavioural differences between High-st and Low-st mink could be found after feeding, whereas High-st mink performed more stereotypies and spent more time performing normal locomotion out in the cage before feeding than did Low-st mink. This could indicate that appetitive behaviour caused by feeding motivation is part of the normal and stereotyped activity observed in High-st mink before feeding.
The behavioural changes during anticipation of a positive or negative reward differed in the mink, indicating the potential of developing an anticipatory test for mink. However, the levels of anticipatory responses were influenced by the feeding and the test situation. The tendency of the animals to reduce stereotypies during anticipation of a reward may primarily depend on the observer’s capacity to direct normal behaviour.

References

Bildsøe, M., Heller, K.E., Jeppesen, L.L., 1991. Effect of immobility stress and food restriction on stereotypies in low and high stereotyping female ranch mink. Behavioural. Processes. 29, 179-189.

Broom, D.M., 1993. Stereotypies as animal welfare indicators. In: Schmidt, D. (Ed.), Indicators Relevant to Farm Animal Welfare. Martinus Nijhoff, The Hague. pp 81-87.

Jeppesen, L.L., Heller, K.E, Bildsøe, M., 2004. Stereotypies in female farm mink (*Mustela vison*) may be genetically transmitted and associated with higher fertility due to effects on body weight. Applied Animal Behavioural Science. 86, 137-143.

Mason, G.J., 1991. Stereotypy: a critical review. Animal Behaviour. 41, 1015-1038.

Mason, G. & Latham, N.R., 2004. Can’t stop, won’t stop: is stereotypy a reliable animal welfare indicator? Animal Welfare. 13, 57-69.

Mittelman, G., Jones, G.H. Robbins, T.W., 1991. Sensitisation of amphetamine-stereotypies reduces plasma corticosterone: implication for stereotypy as a coping response. Behav. Neural. Biol. 56, 170-182.

Moe, R.O., Kingsley-Smidt, H., Kittilsen, S., Bakken, M., 2003. How can happiness be measured in farmed foxes? NJF’s Subsection for Fur Animals, Proceeding. NJF-Seminar no. 354., Lillehammer, Norway, 8-10 October.

Van den Bos, R., Meijer, M.K., van Renselaar, J.P., van der Harst, J.E., Spruijt, B.M., 2003. Anticipation is differently expressed in rats (*Rattus norvegicus*) and domestic cats (*Felis silvestris catus*) in the same Pavlovian conditioning paradigm. Behavioural Brain Research. 141, 83-89.

SAS Institute Inc. 1996. SAS/STATR Software: changes and Enhancement through Release 6.11, Cary, NC. 1104 pp.

Spruijt, B.M., van den Bos, R., Pijlman, F.T.A., 2001. A concept of welfare based on reward evaluating mechanisms in the brain: anticipatory behaviour as an indicator for the state of reward systems. Applied Animal Behaviour Science. 72, 145-171.

Vinke, C.M., van den Bos, R., Spruijt, B.M. Anticipatory activity and stereotypical behaviour in American mink (*Mustela vison*) in three housing systems differing in the amount of enrichment. Applied Animal Behaviour Science (in press).

Wiepkema, P.R., 1987. Behavioural aspects of stress. In: The biology of stress in farm animals. (Ed. By P.R.Wiepkema & P.W.M. Van Adrichem), pp. 113-134. Dordrecht: Martinus Nijhoff.

I – 9 RP

Have fur bearers become domesticated (behavioural and brain biochemistry aspects).

O.V. Trapezov, N.N. Voitenko, V.A. Kulikov
Institute Cytology & Genetics, Siberian Department, Academy of Sciences of Russia; 630090, Novosibirsk, RUSSIA. Fax: 7 (3832) 33 12 78; Tel: 7 (3832) 33 05 12;
E-mail: trapezov@bionet.nsc.ru (*work*), *E-mail*: trap@philosophy.nsc.ru (*home*)

Abstract
It is well known that the wild individuals differ in compare with domesticated in degrees of body conformation, coat colour and correlated behaviour. Minks were tested for behaviour by "*hand catch test*". As a result, two types of minks were distinguished, showing domestic or nondomestic behaviour. As shown the highest number of minks with domestic behaviour occurred among the *Sapphire* colour phase, than *Standard*. Two lines of standard minks were developed through behaviour-targeted selection for 15 years: one showing domestic behaviour, the other nondomestic. In minks showing nondomestic response to human, the level of *serotonin* in the hypothalamus and corpus striatum *was reduced.* This raised the question, what may be the specific differences in brain biochemistry between the *Standard* and *colour phase minks*? It was found that the activity of MAO A, the enzyme of serotonin catabolism, was significantly higher in the brain of *Sapphire* and *Silver-blue* minks than Standard.

Introduction
When we compare the wild individuals with domesticated of the same species, one of the first points which strikes is, that they differ from each other to different degrees in body conformation coat colour and correlated behaviour. This is also true for the American mink that has been captive-bred (under domestication) for more than a century. Its number keeps decreasing in nature, making apparent the growing need in its man-provided welfare. The purpose of the work is to demonstrate that mink has become a domesticate and as such will further benefit from human care.
We proceeded from the development of specific estimates of domestic behaviour in mink, using the "*hand catch test*".
The results of many years of research conducted at the Institute of Cytology and Genetics (Novosibirsk, Russia) showed that mink behaviour can be modified from wild to domestic provided that the experimental (starting) population includes individuals that are more docile, tameable, amenable to domestication than others a major evidence for the capacity of wild mink to become domesticates was the inherited reorganization of behaviour through breeding in captivity, or the reorganization of Nondomestic behaviour to Domestic.
Support that domestic behaviour has genetic bases came from the experimental fur farm of this Institute.

Materials and Methods
Testing the Level of Defensive Behavior Towards Man
Minks were tested for behaviour by "*hand catch test*". As a result, two types of minks were distinguished, showing Domestic or Nondomestic behaviour.
The expression of **Nondomestic behaviour** varied qualitatively enabling to score it. Four scores were assigned to Nondomestic behaviour:
–Score 1. *Fearful response towards human.* When attempts were made to catch the caged mink, it retreated, hid in its wooden kennel, gaping and baring its teeth, cried shrilly or hissed, its posture showed intense emotional stress.
–Score 2. *Attack from the wooden kennel.* When attempts were made to catch the caged mink, it jumped to the entrance of wooden kennel, hid in it to attack the gloved hand, bit it with considerable intensity.
–Score 3. *Active attack outside shelter.* When attempts were made to catch the caged mink, instead of hiding, promptly attacked the hand. Even after the test was over, it kept crying, gnawed in fierce assault the bars of the cage at the sight the approaching gloved hand.
–Score 4. *Attacks enhancing in response to human approach.* Before test onset, i.e. before the breeder opened the cage and stretched out his hand, the caged mink vehemently responded to human presence by about the cage, gnawing its bars.

The expression of **Domestic behaviour** also varied qualitatively allowing to score it. Six scores were assigned to Domestic behaviour:

- *Avoidance of contact* with the gloved hand. This behaviour was assigned score "0". When attempts were made to catch, it turned aside (slowly or rapidly).
- Score + 1 *Demonstration of exploratory responses*. The caged mink calmly responded to the stretched hand, showed the exploratory response, sniffing the hand with quivering vibrissae.
- Score + 2. *Calm response to contact with hand.* The mink displays exploratory reaction when observer bring the tips of his fingers into physical contact with snout and throat. Score +2 differed from those assigned score +1 in that at shorter distances from the hand, they did not retreat from it, endured the contact, allowing to touch face, chest, paws.
- Score + 3. *Active contact shown by the tested mink.* When human approached the cage, the mink excitedly ran around, awaiting human contact, tried to thrust its face out of the bar to reach the approaching hand, infrequently "cooing". When the cage was opened, the mink got up, leaning against the open door, reached out for the gloved hand. Inside the cage, it actively sniffed about the gloved hand, not infrequently leaned on it. When attempts were made to touch any part of its body, the mink dodged and freed itself.
- Score + 4. *The caged mink allowed to touch any part of its body.* It was actively exploratory, played with the hand, but resisted attempts to handle it.
- Score + 5. *The caged mink allowed to handle it.* These were unique among the farm population. They showed extreme domestic behaviour, allowed handling without displaying fear, aggression. Females tolerated examination of her kits, even during lactation in a way making all precautions unnecessary.

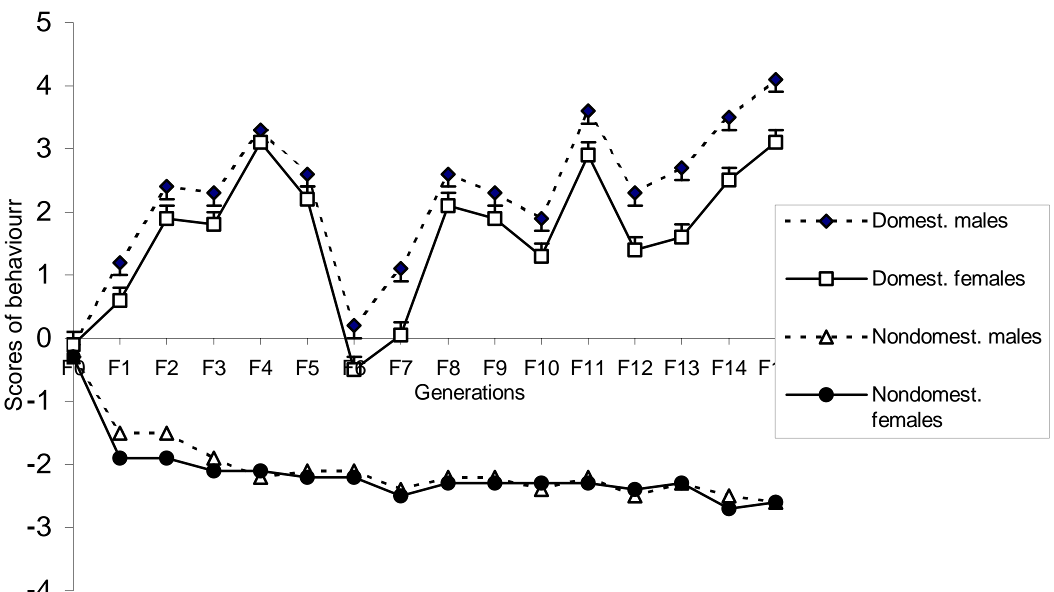
Results and Discussion

As the data in table 1 show docile, tameable, or amenable to domestication minks occur in populations of the standard commercial farms. Most minks of the farm population showed domestic behaviour according to the results of the "*hand catch test*".

Table 1. Coat colour and occurrence frequency of American minks showing domestic behaviour in a farm population

				Nondomestic (*17.3%*)				Domestic (*82.7%*)					
				–4	–3	–2	–1	0	+1	+2	+3	+4	+5
STANDARD	♂	*n*	8 700	6	113	426	559	7262	188	88	41	15	2
		%	*100*	*0.07*	*1.3*	*4.9*	*6.4*	*83.5*	*2.1*	*1.0*	*0.5*	*0.2*	*0.02*
	♀	*n*	8 800	14	214	643	844	6 917	91	41	28	5	3
		%	*100*	*0.1*	*2.4*	*7.3*	*9.7*	*78.6*	*1.0*	*0.5*	*0.3*	*0.06*	*0.03*
	♂+♀	*n*	17 500	20	327	1069	1403	14179	279	129	69	20	5
		%	*100*	*0.1*	*2.0*	*6.1*	*8.0*	*81.0*	*1.6*	*0.7*	*0.4*	*0.1*	*0.03*
SAPPHIRE	♂	*n*	7 200	7	81	318	413	5782	261	138	120	57	23
		%	*100*	*0.1*	*1.1*	*4.4*	*5.8*	*80.3*	*3.6*	*1.9*	*1.7*	*0.8*	*0.3*
	♀	*n*	7 220	17	248	716	945	5071	123	42	43	8	7
		%	*100*	*0.2*	*3.4*	*9.9*	*13.2*	*70.2*	*1.7*	*0.6*	*0.6*	*0.1*	*0.1*
	♂+♀	*n*	14 420	24	329	1034	1358	10853	384	180	163	65	30
		%	*100*	*0.2*	*2.3*	*7.2*	*9.4*	*75.3*	*2.7*	*1.2*	*1.1*	*0.4*	*0.2*

Fig. 1. Change in the mean score for behaviour in the offspring of minks selected for aggressive and tame behavior for 15 generations.



Are the *colour phase minks* more domestic in terms of behaviour than the *Standard*?
This table 1 also shows that the highest number of minks with domestic behaviour occurred among the *Sapphire* colour phase. Thus, the number of minks scored + 5 was tenfold greater among *Sapphire* than *Standard* minks.

ENHANCEMENT OF DOMESTIC BEHAVIOUR AND OF THE REVERSE NONDOMESTIC BY SELECTION

Two lines of standard minks were developed through behaviour-targeted selection for 15 years: one showing Domestic behaviour, the other Nondomestic.
The founding stoks selected for domestic and nondomestic behaviour was of a farm population, 10,000 individuals in size. The F_0 designed to be selected for nondomestic behaviour showed distinct aggressiveness (–2.4 ± 0.1 scores for males and –2.2 ± 0.1 scores for females); and the F_0 selected for docility toward human was scored + 3.6 ± 0.1 for males and + 2.3 ± 0.1 for females. A hundred fifty females and 100 males were taken as the founding generation for selection in either direction. Fig. 1 present the results for 15 selected generations.
Because sexual dimorphism was observed for the selected mink populations, the data for the behavior of males and females are given separately.

The data primarily demonstrate the efficacy of selection for enhanced domestic behaviour and in the reverse direction, for enhanced nondomestic. The mean effect of selection for nondomestic behaviour was manifested in the first two generations (fig. 1), the mean value changed slightly in the subsequent. The course of selection for domestic behaviour was more complicated. The effect was marked up to the fourth generation, the mean value reduced somewhat at the fifth and sharply fell at the sixth so that the initial state almost completely recovered (fig. 1). In the subsequent selected generations, the mean value again rose substantially.
It should be noted that in calculation of the means for the observed response to selection per generation, there were no differences in selection direction, although the effect was strongest for the domesticated males. This was possibly because the selection coefficient for enhanced domestication was smaller in females than males. The mean response to selection for nondomestic behaviour was somewhat smaller and there were no differences between the sexes. However, in the populations selected for nondomestic behaviour, all the offspring was already aggressive in the third generation. Furthermore, aggressiveness smoothly increased in offspring of nondomestic aggressive minks (from – 1.5±0.1 in the F_4 to – 2.2±0.04 in the F_{15} for males and from – 1.9±0.1 in the F_1 to –

2.2± 0.02 in the F_5 for females). This was not observed in the course of selection for domestic behaviour. However, among minks selected for domestication in all the selected generations, there appeared all the two behavioural types: domestic and nondomestic. As seen in fig. 1 selection effect reduced at generations 5 and 6, there appeared many individuals with the nondomestic aggressive response, more among females. From generation 7, the mean domestication score rose again. In contrast to selection for nondomestic behaviour, sexual dimorphism was retained in behaviour expressivity throughout the 15 selected generations. What is more important: the value of the phenotypic variance of the character in the lected for nondomestic behaviour.

The materials for behavioural modification through selection for domestic and nondomestic behaviour reveal heritable variation in the behaviour of farm-bred mink populations. The presence of this adaptive polymorphism is a prerequisite for successful genetic adaptation of minks to captivity conditions and for their historical domestication.

To provide a basis for polymorphism study, a founding stock of minks showing extreme domestic and nondomestic behaviour had to be set up. These were to be subjected to selection in the opposite directions. Thousands of minks had to be analyzed. It was expected to involve young minks of the year. This was because young growing minks were numerous and diverse. To cover diversity wider, one representative of each litter was analyzed. For this reason, parameters of similarities between the parental pairs were not statistically estimated. Based on these estimates, judgements could be made about the range of genetic diversity. However, standard conditions of cage maintenance at the farm and the same limited contacts with human allowed us to believe that the revealed polymorphism had a genetic basis. This was confirmed by the effects obtained in the early selected generations.

The observed heretability expressed as the ratio of the shift produced by selection (R) to the selection differentiation (S) yielded a rather rough estimate (table 2). It indicated, however, that 60% of additive diversity contribute to selection for domestication and about 40% to selection for nondomestic behaviour (37% of females and 38% of males) in two generations. The considerable shift obtained in selection of the first two generations for nondomestic behaviour demonstrated that the number of major genes controlling the threshold nondomestic behaviour was small, one or two, and the genes rapidly became fixed at the early steps of selection. This supported the results of the previous genetic analysis of dog behaviour (*Krushinsky*, 1938, 1945, 1946).

As seen in fig. 1, selection for domestication followed a more complicated course. Judgments were made on the observed heritability (table 2). Additive genetic effect was not responsible for the recovery to the almost initial state observed in generations 4-6. This recovery was hard to explain, although dissimilar responses to selection was a feature of many breeding experiments (*Falconer,* 1960). Quite possibly, maternal embryonic effects started to play an important role in the determination of a character. It is generally accepted that various prenatal factors affect many behavioural traits, including emotional reactivity and the fear response (*Trut, Borodin,* 1976). The extent to which the behavioural phenotype is modified by interference in the prenatal period is dependent on many factors, including the offspring and maternal genotype, the stage of embryogenesis, interference time.

Table 2. Estimates of the observed heretability in selection for domestic and nondomestic behaviour.

Generations	$h^2 = {}^R/_S$			
	Domestic		Nondomestic	
	♀♀	♂♂	♀♀	♂♂
$F_2 - F_0$	0.37	0.38	0.60	0.61
$F_4 - F_2$	0.30	0.18	0.07	0.18
$F_6 - F_4$	–1.43	–0.70	0.05	0.03
$F_8 - F_6$	0.40	0.39	0.06	0.02
$F_{10} - F_8$	–0.10	–0.15	0.01	0.01
$F_{12} - F_{10}$	0.30	0.22	0.01	0.01
$F_{15} - F_{12}$	0.44	0.42	0.02	0.01

However, it may be thought that these interferences have been established by natural selection and act as mechanisms of phenotypic stabilization of a character. There is evidence indicating that the prenatal effect of the maternal environment are reverse to the maternal additive genetic effects, i.e. the maternal genotype determines the low level of a character, whereas mothers promote the formation of its higher level. Thus, prenatal effects may smoothen genetic differences at the phenotypic level. Very likely, by the fourth generation selected for domestication, certain neurochemical mechanisms regulating development are modified by various agents acting on pregnant.This possibility prompted us to further search for differences in the brain serotonin and catecholamine systems between nondomestic and domestic minks.

Interesting relations between selection direction and variability in behaviour were revealed in the course of selection for domestic and nondomestic behaviour. Phenotypic variability in selection for domestication is several times greater than that in selection for nondomestic (fig. 1). This pattern was observed even in the unselected commercial population: phenotypic variability in the expressivity of domestic behaviour considerably surpassed that of nondomestic behaviour. Variability in the expressivity of domestic behaviour is particularly high among *Sapphires* direcessives, which are produced by a combination of two gray colour variations: *aleutian* (genetic symbol for colour type – *a/a*) and *silver-blue* (genetic symbol for colour type – *p/p*). There is a possibility that selection of *Sapphire* minks for domestication was more efficacious than for *Standard.* The coat colour genes have been called with good reason “*the domestication genes*” (*Keeler,* 1942, 1947). A plausible explanation for this are that the early steps are common in the synthetic pathways of the neural mediators and the pigment melanin in minks under domestication.

The materials for behavioural modification through selection for domestic and nondomestic behaviour reveal heritable variation in the behaviour of farm-bred mink populations. Thus, the demonstrated behavioural diversity in mink farms has a genetic nature and the degree of its expression can be estimated by the “*hand catch test*”. The presence of this adaptive polymorphism is a prerequisite for successful genetic adaptation of minks to captivity conditions and for their historical domestication.

The affirmative answer was provided by the “*hand catch test*” results.

As shown in Tab. 1, *Sapphire* (genetic symbol for colour type, *a/a p/p*) raised in the usual commercial farms are more domesticated than *Standard* (colour type +/+). There was good reason for referring to the coat colour genes as the domestication genes (*Keeler*, 1942, 1947). Therefore, it follows that the *colour phase* resulting from breeding mink for many generations in captivity is more domesticated, in terms of behaviour, than *Standard*.

This raised the question, what may be the specific differences in brain biochemistry between the *Standard* and *colour phase minks*?

Do changes in brain biochemistry take place in the course of mink domestication?

Genes that influence the extent to which the emotional state is manifest have been identified. These genes program the synthesis of *serotonin* involved in the conveyance of neural impulses in the brain structures and of the *enzyme monoaminoxidase* degrading serotonin. It has been amply demonstrated that *serotonin* is important to the neuroregulation of behaviour (*Naumenko & Popova,* 1975; *Popova et al.,* 1978).

Interest in serotonin keeps augmenting with reference to the problem of the domestication of fur bearers. This is because serotonin participates as an inhibitory factor in the central regulation of different types of aggression. The higher level of brain serotonin in domestic animals can make them less aggressive. Previously was shown the activity of the key enzyme of serotonin biosynthesis *tryptophan hydroxylase* was substantially lower in the midbrain of highly aggressive foxes than in domestic (*Popova et al.,* 1975). This might have played a role in the domestication of wild fur animals, and the reorganization of their behaviour and many of their morphological-physiological changes might have been the result of the same genetic changes produced by *natural selection in captivity* vectorized for the amenability to domestication (*Belyaev*, 1969, 1979, 1981).

In fact, the inhibitory properties of serotonin have been demonstrated for different models of aggression (*Popova et al.,* 1978; *Malmkvist et al.,* 2003).

This raised the question, if there exists in the founding population of farm bred minks not subject to selection polymorphism for brain monoamine

acids and, if it does exist, how is it related to animal response to human?
It was found that:

1. The farm bred mink population was heterogeneous with respect to the activity of the brain monoergic systems. This polymorphism was consistent with the one observed for the response to human. Minks with domestic and nondomestic response to human all differed by the measured characteristics of the serotonin and catecholamine brain systems.
2. In minks showing nondomestic response to human, the level of *serotonin* in the hypothalamus and corpus striatum *was reduced* (Fig. 2) and so was the content of its metabolite 5-hidroxyindolactic acid in the corpus striatum (Fig. 3).

Materials and Methods

Brain monoamines were studied in minks differing by the response to human. There were 30 male American minks (*Standard,* +/+), aged 6 months, of a common farm population that has been, as yet, not selected for behavioural response to human. They were scored as follows: nondomestic – 3.6±0.2; domestic 0.0±0.0; domestic + 4.1±0.1. There were 10 minks in each group.
The experiments were performed in November at pelting for commercial purposes. The minks were sacrificed by cervical dislocation, the brain was placed on ice, and the midbrain, hypothalamus and corpus striatum were dissected. The level of noradrenaline, dopamine, serotonin and of its main metabolite 5-hydroxyindolacetic acid (5-HIAA) were determined fluoremetrically (*Jacobowitz, Richardson,* 1978). The level of dopamine was determined in the corpus striatum; the content of noradrenaline, serotonin, and 5-HIAA was measured in the hypothalamus, midbrain (with the pons area), and corpus striatum.

Results

It was found that serotonin content varies in the brain structures of minks differing by the response to human. In the hypothalamus and corpus striatum, the content was lower in nondomestic minks than domestic (fig. 2, 3). The content of the serotonin metabolite 5-HIAA in the corpus striatum was also lower in nondomestic minks compared to evasion . No difference were found in the content of serotonin and 5-HIAA in the corpus striatum in minks of all three groups.

INFLUENCE OF GENES AFFECTING COAT COLOUR ON SEROTONIN TURNOVER IN THE MINK BRAIN.

There is evidence to suggest that the catabolism of brain neuromediators is also altered in minks carrying genes affecting coat colour (*Voitenko & Trapezov,* 1999). As the data in table 1 show, genes affecting coat colour act like the domestic behaviour genes. These observations are consistent with *Keeler* (1947) who justly called the coat colour genes “*the domestication genes*”. This prompted us to compare brain serotonin metabolism in *Sapphire* and *Standard* minks of a farm population not subject to selection targeted at domesticated behaviour.

Fig. 2. The level of serotonin in the brain regions of minks differing by the response to human.

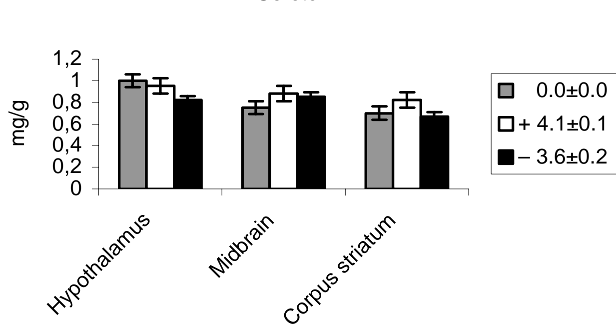


Fig. 3. The level of 5-HIAA (serotonin metabolite) in the brain regions of minks differing by the response to human.

5-hydroxyindolacetic acid

mg/g

Hypothalamus Midbrain Corpus striatum

The measurements in the brain areas included: 1) the levels of serotonin and of its major metabolite 5-HIAA; the activities of the metabolic enzymes of serotonin; 2) the key enzyme of serotonin biosynthesis tryptophan hydroxylase, and of the

major catabolic enzyme monoamine oxidase type A (MAO A).

Materials and Methods

Male mink were used in the experiments. Their genotypes were: 1) *Standard* (+/+), 2) *Sapphire*, color phase homozygous for the *aleutian* and *silver-blue* coat colour genes (*a/a p/p*), and 3) *silver-blue*, homozygous for the coat colour gene (*p/p*). The experiments were performed in November at pelting for commercial purposes. The mink were sacrificed by cervical dislocation, the brain was placed on ice, and the midbrain, hypothalamus and corpus striatum were dissected. The levels of serotonin and 5-HIAA were determined fluorometrically (*Jacobowitz, Richardson,* 1978); the measured values were expressed as µg/g brain issue. The activity of tryptophan hydroxilase was determined fluorometrically (*Kulikov*, 1992) in the presence of 6, 7-dimethyltetrahydropteridine co-factor; the measured values were expressed as nmoles of 5-hydroxytryptophaaan/mg protein/min. The activity of the monoamine oxidases A type was determined by spectrophotometry (*Gorkin*, 1981) in the presence of serotonin (substrate of MAO A) and benzylamine.

The kinetic parameters K_d and V_{max} were estimated using the least squares method (*Cornish-Bowden*, 1979). Student’s t-test was applied to treat the other parameters.

Results

This analysis demonstrates that *Sapphire* colour phase considerably affected serotonin metabolism in the brain (Fig. 4).

It was found that the activity of MAO A, the enzyme of serotonin catabolism, was significantly higher in the brain of *Sapphire* and *Silver-blue* minks than Standard (Table 3).

An increase in the activity of the enzyme of serotonin biosynthesis was found in the midbrain of *Sapphire* mink (fig. 5). It may be concluded that the changes in the activity of the key enzyme of serotonin biosynthesis tryptophan hydroxylase are caused by the *Silver-blue* colour phase.

Fig. 4. Concentration of serotonin and its metabolite 5-hydroxyindolacetic acid (5-HIAA) in the midbrain of *Standard*, *Sapphire* and *Silver-blue* mink.

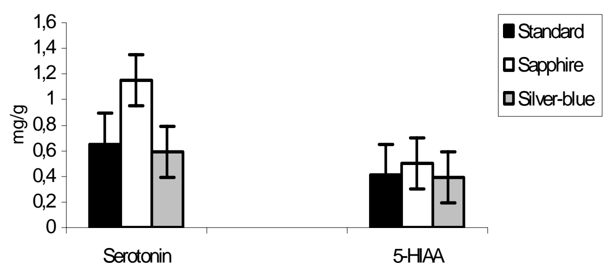


Table 3. MAO A activity in the midbrain and hypothalamus of *Standard*, *Sapphire* and *Silver-blue* mink

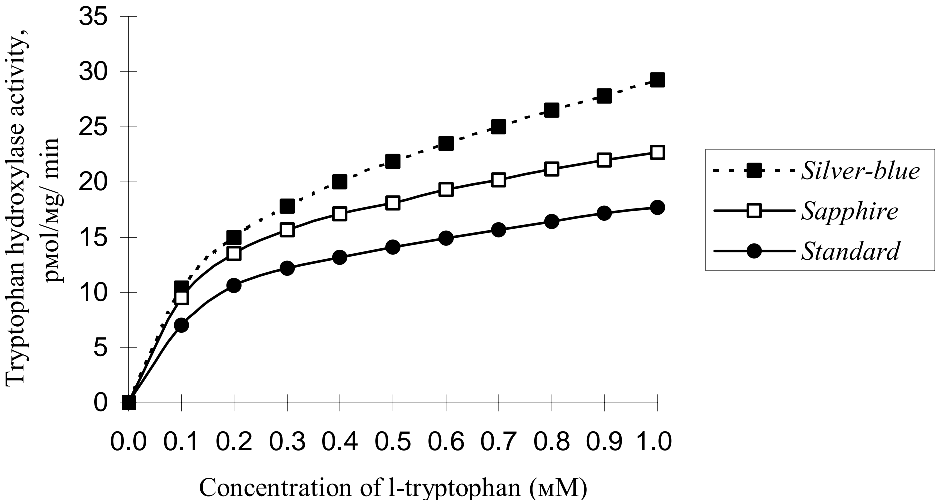
Colour phase	Number of animals	The activity of type A MAO A	
		Midbrain	Hypo-thalamus
Standard	10	2.17± 0.32	3.51± 0.62
Sapphire	10	3.05± 0.23*	2.41± 0.24
Silver-blue	14	4.84± 0.30**	2.59± 0.29

* *p* < 0.05
** *p* < 0.01 in comparison with *Standard* mink (*Student’s “t” test*).

Significant changes in serotonin metabolism were demonstrated in more domesticated *Sapphire* mink. The pleiotropic effect of such colour phase is manifest as changes in the major metabolic enzymes of serotonin, i.e. the key enzyme of serotonin biosynthesis, tryptophan hydroxylase, and the catabolic enzyme MAO A.

Serotonin is a phylogenetically ancient brain neurotransmitter with an extremely wide spectrum of action (*Naumenko & Popova*, 1975) and for this reason at least some of the physiological features of mutant colour phase mink may be explained by changes in serotonin metabolism. One of these features may be reduced nondomestic behaviour of *Sapphire* mink towards humans.

Fig. 5. The tryptophan hydroxylase activity in the midbrain and hypothalamus of different colour phase mink.



References

Belyaev D.K. 1969. Domestication of animals. Science. P. 47–52.

Belyaev D.K. 1979. Destabilizing selection as a factor in domestication. Journal of Heredity 70:301–308.

Belyaev D.K, Trut L.N. 1981. Accelerating evolution. Science in USSR 5:24–64.

Castle W.E. and Moore L. 1946. Mutations of mink under domestication. J Hered 37:137-145.

Falconer D.S. Introduction to Quantitative Genetics. 1960. Oliver and Boyd. London

Gorkin V.Z. (1981). Amine oxidases and their role in medicine. Moscow, Medicina (In Russian).

Keeler C.E. 1942. The association of the black (non-agouty) gene with behavior in the Norway rat. Journal of Heredity. 33:371-384.

Keeler C.E. 1947. Coat color, physique, and temperament. Journal of Heredity 9:271–277.

Krushinsky L.V. Study on behaviour phenogenetics in dogs. Biological Journal, 1938, vol 7, № 4, P. 869-890 (In Russian).

Krushinsky L.V. The role of inheritance and training conditions in the manifestation and expression of behavioural traits in dogs. Proceedings of the USSR Academy of Sciences. Biological Series, 1948, № 1. P. 69-81 (In Russian).

Malmkvist J., Hansen S.W., Damgaard B.M. 2003. Effect of serotonin agonist buspirone on behaviour and hypothalamic-pituitary-adrenal axis in confident and fearful mink. Physiol. Behav. V. 78. P. 229-240.

Jacobowitz D.M., Richardson J.F. 1978. Method for rapid determination of nor-epinephrine, dopamine, and serotonin in the same brain region. Pharmacol. Biochem. Behav. Vol. 8 (5):515-519.

Popova N.K., Voitenko N.N., Trut L.N. 1975. Change in the content of serotonin and 5-hydroxyindolacetic acid in the brain during selection of silver foxes for behaviour; Reports of the USSR Academy of sciences, 223, № 6, p. 1496-1498 (In Russian).

Popova N.K., Naumenko E.V., Kolpakov V.G. 1978. Serotonin and behaviour. Novosibirsk: Science, 303 p. (In Russian).

Popova N.K., Voitenko N.N., Pavlova S.I., Trut L.N. 1980. Genetics and phenogenetics of hormonal characteristics in animals. VII. Relationship between brain serotonin and hypothalamo-pituitary-adrenal axis in emotional stress in domesticated and nondomesticated silver foxes. Genetica. V. 16. № 10. P. 1865-1870 (In Russian).

Smith S.E., Whitaker C.H., Davis L.F., Nobel P.V. 1941. The inheritance of three coat color mutations in ranch-raised mink. J. Hered., 32, 173-176.

Trut L.N., Borodin P.M. The formation of behaviour in certain laboratory rodents under environmental effect during different stages of development. Advances in Modern Biology. 1976, vol. 82, issue I (4), p. 143-155 (In Russian).

Voitenko N.N., Trapezov O.V. 1999. Species-Specifisity of Brain Monoamine Oxidases in Mink and Rat. Zh. Evol. Biokhim. Fiziol., vol. 35, № 6, p. 462-465 (In Russian).

Voitenko N.N., Trapezov O.V. 2001. Effect of Four Coat Color Mutations (C_R, S, S^H, and h) on Brain Monoamine Oxidase in Mink (Russian Journal of Genetics, Vol. 37, No. 5, pp. 617-620).

I – 11 P

Evaluation of comfort of fur-bearing animal keeping by analyzing behaviour

Igor A. Plotnikov, Olga Ye. Yevenko, Oleg Yu. Bespyatykh
Russian Research Institute of Game Management and Fur Farming, Russian Academy of Agricultural Sciences, 79 Engels Street, Kirov, 610000, Russia
e-mail: bio.vniioz@mail.ru

Abstract
Conditions of fur-bearing animal keeping in different cages: generally used and those constructed according to the recommendations of the Council of Europe were studies. The welfare of animal keeping was evaluated by behaviour. The methods of finding, registration and analysis of the elements of fur-bearing animal behaviour were worked out. Animals' behaviour was observed in two ways: visually and with video cameras. The latter way is more universal and makes it possible to observe animals without disturbing them in their covers and in a dark time of a day. The main studies were carried out on red fox. The methods were tested on polar fox, raccoon dog, marmot, nutria, sable, mink, ferret. 170 elements of behaviour were totally observed, 35 of them were used most often. The analysis of behaviour will give an opportunity to evaluate the comfort of keeping conditions, the efficiency of using a new way or element of technology of fur-bearing animal keeping.

Introduction
In 1999 the Standing Committee of the European Convention for the Protection of Animals Kept for Farming Purposes under the Council of Europe adopted “Recommendations Concerning Fur Animals – T-AP (96) 19”. Requirements on significant improvement of the conditions of animal keeping given in those recommendations should be fulfilled by the 31st of December 2010. Besides, certain organizations that support the protection of animals stated that conditions of fur-bearing animal breeding were not humane, and drastic measures should be undertaken to improve the welfare of animal keeping.
Some requirements advanced are not scientifically grounded, so it is necessary to study their necessity in details. When animals do not show abnormal behaviour, self-wounding and diseases then it means physical and mental health and may be interpreted as comfort keeping (Niedzwiadek et al., 1998). The indices of comfort keeping can not be objectively defined only by traditionally used biochemical and morphological studies of animal health. Environmental conditions don't always have a direct effect on physical and morphological features. The change of metabolic and behaviour reactions of an organism is most usual (Schwarz, 1960). Changes in behaviour are the first reaction of an organism to environmental transformation that is easily found. Comparing those reactions we have an opportunity to realize in what direction conditions of animal keeping change – favourable or unfavourable one (Kovalcikova & Kovalcik, 1974).

Material and Methods
The main studies were carried out on red fox. Observations of behaviour of other species of fur-bearing animals (polar fox, raccoon dog, marmot, nutria, sable, mink, ferret) were also made. We worked out the method of finding, registration and analysis of caged fur-bearing animals. That method was universal and suitable for every species of animals but the features specific for them should be taken into account. Behaviour was evaluated in two groups of animals simultaneously. The first group served as a control one and animals of that group were kept under usual conditions. The animals of the second group (an experimental one) was kept under new conditions that were supposed to be more comfortable (microclimate conditions, the size of a group, the area of a cage, a cover, a shelf for rest, toys and so on). The number of cages in every group for observing the behaviour per one investigator made up 4 cages for red fox, polar fox, raccoon dog, marmot, nutria and sable and 6 ones for mink and ferret.
The registration of the elements of behaviour was carried out by the observer and with video cameras with an infrared light that were connected with a monitor and a recording device. 1-2 video cameras set up at some distance from a shed, and with video cameras – peep-holes set up in the roof of a house were used. Video observation was the only way to record animals' behaviour in covers and in a dark time of a day. Cameras were switched on for 1 minute at intervals of 5-15 minutes. The whole

information was entered in the ethological observation record. For every element of behaviour its own number and graphic symbol was given. With computer statistical processing the frequency and duration of showing of different elements of behaviour were evaluated.
All forms and elements of behaviour were subdivided into groups, two of them were the principal ones – a comfort and discomfort behaviour. Such classification and analysis of behaviour as to the comfort level made it possible to give a well-reasoned conclusion on the efficiency of using a new version of caged keeping of fur-bearing animals.
Studying of the influence of microclimate on the behaviour was carried out on red fox. Together with behaviour registration the changes in microclimate indices were registered. They were temperature, relative humidity, air speed, pressure (Plotnikov at al., 2000). Observations were carried out in summer and winter as those seasons of a year were characterized by the highest positive and negative fluctuations of microclimate indices and had the most significant influence on the changes of animal behaviour.

Results and Discussion

170 elements of behaviour were totally revealed, 35 of them were observed most often.
Elements of behaviour associated with taking care of a body were referred to a comfort one. In the majority of animal species the way of maintaining the cleanliness of a body was expressed in different ways of fur licking, scratching, shaking. Such behaviour was observed more often and longer during the period before and after sleep. Different ways of scratching and shaking oneself were observed at feeding, locomotion and rest. Yawning and stretching oneself took place after sleep.
Elements of behaviour similar to comfort were referred to a separate group. They were different poses of local activity (poses at which animals are awake, being on one and the same site and in a sitting, standing or lying position).
Elements of behaviour characterized as discomfort were included in a separate group. They were different showing of nervousness (fear, aggression, pain, depression and so on). Discomfort had a pronounced outward exhibition. If an animal was aggressive, then it had raised ears, fixed look directed forward, disheveled hair on the back and in the neck, a strainedly raised tail. Depression was characterized by the decrease of intensive activity and appetite. A head, a tail and flabby cheeks and eyes became lackluster. Even a very low level of fear was invariably expressed in the behaviour when a tail was put between legs. That putting of the tail between legs was accompanied with drawing ears backward.
A greater influence on the behaviour of animals was caused by microclimate changes. Investigations carried out on red fox and other representatives of *Canidae* showed that with the rise in temperature of environmental air up to 20 °C a motor activity of animals increased. Animals became more active, different elements of playing behaviour and jumping to a mesh wire cage side were observed. When the rise of temperature continued a typical pose of rest was noted when an animal lay on its side flat on the floor. And its pads were at a distance from a body. Therefore, animals instinctively tried to increase heat emission enlarging their body area. When it was too heat (30 °C and higher), and in particular at the direct sun radiation a mouth cavity was open, a tongue dangled, the breathing became faster. That favoured the cooling of an organism through the increasing of an evaporation rate from mucous membranes.
If the effect of high temperature took place for a long time (usually in mid-summer) the appetite of animals fell, their motions became sluggish and they lay longer. During those periods overheating of organisms might occur, and in more severe cases – even a heat stroke.
All elements of behaviour mentioned may be included in the group of elements disturbing comfort. These forms of behaviour indicate that it is necessary to take urgent measures to improve the technology of keeping of caged fur-bearing animals.
Fur-bearing *Carnivora* bred in farms are adapted to low environmental temperatures most of all. This is associated with the fact that their wild ancestors live in northern latitudes.
In a winter period of a year low air temperatures increase the heat emission in animals, so to conserve a steady temperature of a body in an organism metabolism becomes more intensive. Animals are not active during that period, the greater part of the time they lie rolling themselves up into a ball. Their feet are bent at an angle and are put under their body. A head lies on hips, a muzzle rests against the root of a tail. A tail rounds a body on the outside. Such pose decreases a body surface and heat losses and preserves sensitive parts from overcooling. When the air temperature falls as low as – 30 °C and

lower, reflex shiver in animals is observed because of skeletal muscular contraction. Hair cover becomes disheveled. Such behaviour also shows the state of discomfort.

It is known that at the increased air humidity animals endure worse both high and low air temperatures. A high relative humidity of air results in worsening of an appetite and makes it difficult to evaporate moisture through a respiratory tract. At that time animals become inert, motor activity decreases. At high positive temperature the passes in sheds and cages should not be splashed with water. That will cause the increase of relative humidity of air and worsen the state of animals, and therefore that will immediately change their behaviour.

The rise of the air speed always favoured the welfare of animals at high positive temperatures, and it increased discomfort at negative temperatures. The highest depression in caged red foxes was observed in a winter period at low atmospheric pressure under the effect of cyclone when the air temperature was about 0 °C, relative humidity – 100 %, air speed – 5-8 m/s with rushes of wind up to 14 m/s. Beyond doubt such conditions maximally increased a cooling effect of air and resulted in overcooling of young and weakened animals.

Conclusion

Therefore, when analyzing behaviour of animals, revealing and registrating elements of comfort and discomfort, it is possible to evaluate in time the conditions of animal keeping and to suggest measures on microclimate optimization. The analysis of behaviour allows to draw substantiated conclusions on the efficiency of using of a new way or an element of technology of caged fur-bearing animal keeping.

References

Kovalcikova, M., Kovalcik, K. 1974. Adaptacia a stress v chove hospodarskych zvierat. Bratislava (in Czech).

Niedzwiadek S., Zaenz Ya, Zon A. 1998. Comfort for fur-bearing animals. Krolikovodstvo i Zverovodstvo, 1: 12 (in Rus.).

Plotnikov I.A., Bespyatykh O.Yu., Gaziziv V.Z., Domski I.A. 2000. Characteristics and optimization of husbandry conditions of herbivorous fur-bearing animals. Scientifur. 24:4:170-171.

Recommendations concerning fur animals – T-AP (96) 19. 1999.

Schwarz S.S. 1960. Certain mechanisms of ecological conditionality of interior peculiarities of terrestrial vertebrates. *In* Problems of flora and fauna of Urals: Proceedings of the Institute of Biology of Ural Branch of Academy of Sciences, 14: 113-178 (in Rus.).

I – 12 RP

Conflicts arise between minks of different behavioural types

O.V. Trapezov, I.N. Oskina, R.G. Gulevich
Institute Cytology & Genetics, Siberian Department, Academy of Sciences of Russia; 630090, Novosibirsk, RUSSIA. Fax: 7 (3832) 33 12 78; Tel: 7 (3832) 33 05 12;
E-mail: trapezov@bionet.nsc.ru (work), E-mail: trap@philosophy.nsc.ru (priv)

Abstract
Hostility between two minks raised in the same cage (♂+♂ and ♀+♀) can be evaluated in dominance terms: “alpha” (α) and “beta” (β) individuals. This is vividly manifest at pelting time in conditions of Siberia when temperature drops to – 30ºC, food freezes hard on cage wire nets, and fur animals lose weight. As a result the leader in the pair weights more than the subleader. The important question then is, how sex and behavior may affect conflict between two paired minks. To study the role of typical behavioral patterns involved in conflict between young minks raised pair wise, standard males and females of groups selected during 15 generations for tame and aggressive behavior in response to human were used. The obtained results (intensity of bites, body weight, cortisol and plasma transcortin levels) shows that aggressive animals suffered more from stressing exposures.

Introduction
Several types of aggression can be in juvenile pair-wise housed mink as based on different motivational backgrounds.

1. Aggression engendered by fear characteristic of cornered animals, the aggression is preceded by attempts to flee.
2. Aggression between males, the aggression is exaggerated when two strange males encounter. To study this aggression type, males are conventionally maintained alone, then in pairs. The preceding isolate has usually a decisive influence on the manifestation of the aggressive response. There are data to indicate that conditions of isolation enhance the trend toward fighting possibly related to a decrease in the levels of the brain biogenic amines serotonin and noradrenaline (*Welch*, 1970). The emergence probability of aggressiveness between males can be reduced by drugs when pharmacological anticholinergic effects (interfering with acetylcholine function) which inhibit isolation produced aggressiveness. However, a meaningful interpretation of data on the influence of drugs on the aggressiveness type is difficult because it can be suppressed by substances that either elevate on lower the level of acetylcholine, serotonin or noradrenaline in the brain.
3. Aggression due to irritation differs from other aggression types in that it is elicited by a variety of stimuli many of which, in all likelihood, are annoying. The hypothalamic ventromedial nucleus and also sex hormones play an important role in aggression of this type.

Hostility between two animals raised in the same cage can be evaluated in dominance terms: one individual can without penalty or attack reprimand another. In these conditions, a despot is brought into prominence, it is the “alpha” (α) individual having priority for food access. The other “beta” (β) individual, is pushed away. This is vividly manifest at pelting time in conditions of Siberia in the second half of October, when it is already frosty, and by November, when temperature drops to – 30ºC, food freezes hard on cage wire nets, and fur animals lose weight. Good heat isolation in wood boxes is frequently too costly. As a result, at the time of pelting, the leader in the pair weighs more than the subleader. The important question then is, how sex and the typical behavioural patterns may affect conflict between two paired minks.

Materials and Methods
This study was performed at the experimental fur farm of the Institute of Cytology and Genetics (Novosibirsk). Minks were tested for behaviour by “*hand catch test*”. As a result, two types of minks were distinguished, showing domestic or nondomestic behaviour. Two lines of standard minks were developed through behaviour-targeted selection for 15 years: one showing domestic behaviour, the other nondomestic (see proceedings of IFASA 2004: *O.V.Trapezov, N.N.Voitenko, V.A.Kulikov:* “Have fur bearers become domesticated (behavioural and brain biochemistry aspects”).

Mink breeding practice demonstrated that it is most profitable to raise young mink in pairs of different cages. To identify the typical behavioural patterns (aggressive and tame) in tolerance to conflict situations, we deliberately used pairs of the same sex. One reason was to exclude, for example, the effect of heavier males on the result of leadership establishment. For this purpose, from weaning to pelting time (November 20th), pairs of the same sex whose weights were the same before grouping were used. The groups were as follows.

1. Pairs homogenous in behavior (aggressive male + aggressive male), (tame male + tame male), (aggressive female + aggressive female), (tame female + tame female).
2. Pairs heterogenous in behavior (aggressive male + tame male), (aggressive female + tame female). Identification (classification) of the tame or aggressive leader was subsequently based on body weight only.

Two-month-old mink males were weaned in June and placed into free cages in pairs simultaneously. The pairs were selected so that the animals were of equal weight; one of them was derived from a mother with the aggressive type of behavior, the other from a tame mother. In the age of seven months the males were tested for behavior and weighed. Further hormonal studies were done on 17 pairs of animals, where the neighbor differed clearly in body mass. Simultaneously, blood samples were taken from the tips of the tails. After slaughter bite marks were counted on the fresh skins. The numbers of animals in the groups are indicated in figure legends.

The levels of total cortisol in blood were tested by the method of competitive protein binding (*Murphy,* 1967), using mink cortisol-binding protein (transcortin), cortisol (Sigma Chemicals, US), and [1, 2, 6, 7-3H]-cortisol 90 Ci/mmol (Isotope, St.-Petersburgh, Russia).

Intra- and interassay coefficients of variation were less than 5% and 10%, respectively. The percentage and level of free cortisol were detected according to *Martin, Cake, Hartmann, Cook* (1977). Transcortin was assayed by the radioligand method (*Tinnikov,* 1993). To assess the precision of this method transcortin was measured in 10 various samples on different days, each time using freshly made up reagents. The mean coefficient of variation was 8%. The dissociation constant K_d, characterizing the binding between transcortin and cortisol, was found by the Scatchard method to be 4.0 nM.

The results are represented as mean values and mean errors. The statistical treatment was done by analysis of variance and the Student test. Because of wide variability of data on the level of free cortisol, the Wilcoxon test was applied for comparison of the mean values.

Results

Based on body weight measurement data, the α -individuals were significantly heavier than the β -individuals in all male or female pairs by the beginning of August. This difference in body weight kept increasing, and maximal discrepancy was reached by pelting time. There were just a few pairs with the same body weight. In pairs heterogeneous with respect to behavior, it was possible to distinguish which behavior type (tame or aggressive) confers body weight advantage. As the data in Tables 1 and 2 show, a weak tendency was observed for males: leaders for body weight proved to be tame and, vice versa, body weight leaders proved to the aggressiveness among female (33 g versus 29 g). When differences in body weight between the α - and β -individuals were compared, it was found that the β -aggressive males lost most weight. Thus, their weight was 80% of the α -individuals in group I, 70% of those in group IV.

Tame β -individuals, when raised in homogenous and heterogenous pairs, suffered smaller losses: 85% in α of group II and 84% in α of group III. Clearly, the β -aggressives suffer most appreciable body weight losses for males raised in heterogenous pairs.

The situation was less dramatic for females. In homogenous for behavior pairs (groups I and II) body weight in both aggressive + tame pairs was 86% of α. In heterogenous pairs, in contrast to males, greatest body weight losses were observed for the β -tame females of group III (82% the β of the α).

At the time of molting, the conflict situation in the pairs became much more dramatic, judging by the frequency of fights leading to increasing number of bites on pelt. The intensity and duration of bites in male pairs was significantly higher than in female pairs.

As a measure of variability in the number of bites, we used variance values which are the values of mean square deviations from the mean value of a character (σ^2) and also the maximum and minimum values for bites.

From the data in Table I it follows that mean bite number per pelt of groups I, II and III is significantly higher than that in the α -males. The highest bite number per pelt was for the β -tame males of group III. In this very group, the smallest number of bites was recorded for the α -aggressive males. As for group IV, it illustrates well the hereditary predisposition of aggressiveness to human in a stressing situation of inadequate food supply. These were extremely aggressive to counterparts, despite significantly smaller body weight. In this group, the β -aggressive males, inferior to the α -tame ones in body weight, (by 423 g lighter) bit them more frequently. How to explain this behavior?

Discussion

Aggressive behavioral patterns are affected by many hormones, the products of endocrine glands (the pituitary, gonads, adrenals). The hormones are addressed at target cells in the brain, modifying the level of the biogenic amines serotonin and adrenaline. The sex hormones determine the potentional ability for aggressive behavior and also maintain aggressiveness in the adult. The products of ovary secretion usually attenuate aggressiveness in females, whilst testicular androgens enhance it in males. This may be a reason why fights among males are fiercer than females. The adrenal cortex releases at least 28 different glucocorticoid hormones contributing to the development of aggressiveness. To examine their effects during pelting time, the functional state of the adrenals was studied. It was found the basal glucocorticoid level in the β -aggressive males of group IV significantly increased when they are chronically stressed, whilst, like in the β -tame females of group III, in the same heterogeneous maintenance, the level remained normal. Data were also obtained indicating that, as a result of long food stress, in the β -aggressive males of group IV, the level of a specific plasma protein transcortin significantly decreased. And transcortin is important for retaining adrenal cortex hormones in bound or inactive state, when food conditions are normal.

Consequently, in conditions of strong competition for food among tame males (both the α and β), in contrast to aggressives, body weight does not considerably affect cortisol and transcortin levels. Perhaps, aggressive males, lighter than their partners, suffered more from stressing exposures not only because the concentration of plasma cortisol is maximal, but also because that of plasma transcortin is minimal. These animals proved to be less stress tolerant to competitive struggle for food compared with tame males.

Numerous hormones, the products of the endocrine glands (the pituitary, gonads, adrenals) serve as chemical signals for relationship among organs. It was found that hormone effect on behavior is dependent on genotype, individual features, time of the year, interaction between individuals, sex, and species-specificity. The sex hormones act also during the neonatal period, determining the potentialities for aggressive behavior and they retain aggressiveness in adulthood. The adrenal cortex secretes at least 28 various steroid hormones influencing metabolism and infection resistance. The hormones are delivered to appropriate target cells in the brain. Their function is manifold. The hormones alter the protein-synthesizing function of DNA and RNA. They can affect behavior, acting on the sensory perceptive mechanisms, on the activity of the nervous system and on the effect or mechanism providing behavioral acts. In the nervous system, hormones exert an influence on morphological structures, on physiological activity and neurotransmitter function. Estrogen, progesterone and testosterone influence brain activity, as evidenced by changes in EEG, evoked brain potentials, and single neuron activity (*Komisaruk,* 1971; *Pfaff et al.,* 1973). Hormones affect the level of monoamines in the brain, which are, in turn, under the influence of their level. Thus, estrogen causes a decrease in noradrenaline concentration in the anterior hypothalamus. It has been suggested that progesterone exerts an inhibitory influence on brain serotoninergic activity.

Glucocorticoids are important as transmitters in the behavioral and psychological effects of stress (*Munch et al.,* 1984). There are ample data indicating that basal glucocorticoid level elevates in conditions of chronic stress (*Schribner et al.,* 1991; *Katz et al.,* 1981; *Cure et al.,* 1989; *Kant et al.,* 1987; *Neufeld et al.,* 1994). Such a prolonged state of high basal glucocorticoid level can produce profound changes in the state of metabolic processes, nervous excitation and animal behavior. The more recent data indicated that chronic stress leads to a decrease in the level of the corticosterone-binding protein, or transcortin (*Neufeld et al.,* 1994). Transcortin is a plasma protein keeping adrenal cortex hormones in a bound or inactive state (*Hammond G.L.* 1990). As a consequence, transcortin-bound cortisol cannot gain access to the needed receptors in the target tissues (*Padridge,*

1981). As high as 90-95% of plasma glucocorticoids are transcortin-bound, with the decreased transcortin level producing a measurable increase in unbound or free biologically active cortisol (*Mendel*, 1989). In this way , an increase in the basal glucocorticoid and a decrease in transcortin levels as consequences of chronic stress lead to a considerable rise in free cortisol and to its subsequent strong effect on steroid hormones in the target tissues. It has been shown that transcortin level decreases also in conditions of stressing starvation (*Tinnikov*, 1993).

Hormones have a significant effect on food consumption and body weight (*Wade,* 1976). Sex differences in food consumption body weight are due to both the organizing role of perinatal and activating effects of hormones on adults. The products of ovary secretion decrease, as a rule, body weight, while androgens increase it.

Interaction of the gonad-pituitary axis is manifest also as changes in body weight. Genes do not affect behavior by “magic”. The pathways from a gene to a behavioral trait are intricate**,** through biochemical, morphogenetic, and physiological, milestones. Changes in behavioral patterns result from the combined action of genes and environment. All this ensures the development of a fit organism.

References

Cure M. 1989. Plasma corticosterone response in continuous discontinuous chronic heat eaposure in the rat. Physiol Behav 45:1117-1122.

Hammond G.L. 1990. Molecular properties of corticosteroid binding globulin and the sex-steroid binding proteins. Endocr Rev, 10:232-246.

Kant G.J., Leu J.R., Anderson S.M., Mougey E.H. 1987. Effects of chronic stress on plasma corticosterone, ACTH and prolactin. Physiol Behav, 40:775-779.

Katz R.J., Roth D.A., Carroll B.J. 1981. Acute and chronic stress effects on open field activity in the rat: implications for a model depression. Neurosci Behav. Rev, 5:247-251.

Komisaruk B.R., Adler N.T., Hutchison L.B. 1972. Genital sensory field: Enlargement by estrogen treatment in female rats. Science, 178, 1295-298.

Martin C.E., Cake M.H., Hartmann P.E., Cook I.F. 1977. Relationship between foetal corticosteroids, maternal progesterone and parturition in the rat. Acta endocrinologica. 84, 167-176.

Mendel C.M. 1989. The free hormone hypothesis: a physiologically based mathematical model. Endocr Rev, 10:232-246.

Munck A., Guyre P.M., Holbrook N.J. 1984. Physiological functions of glucocorticoids in stress and their relation to pharmacological actions. Endocr Rev, 5:25-44.

Murphy B.E. 1967. Some studies of the protein binding of steroids and their application to the routine micro and ultramicro measurement of various steroids in body fluids by competitive protein- binding radioassay. J.Clin. Endocrinol.Metabol. 27, 973-990.

Neufeld J.H., Breen L., Hauger R. 1994. Extreme posture elevate corticosterone in a forced ambulation model of chronic stress in rats. Pharmacol Biochem Behav, 47:223-240.

Padridge W.M. 1981. Transport of protein-bound hormones into tissues in vivo. Endocr Rev, 2:103-116.

Pfaff D., Lewis C., Diakow C., Keiner M. 1973. Neurophysiological analysis of mating responses as hormone-sensitive reflexes. Progress in Physiological Psychology, **5**, 253-297.

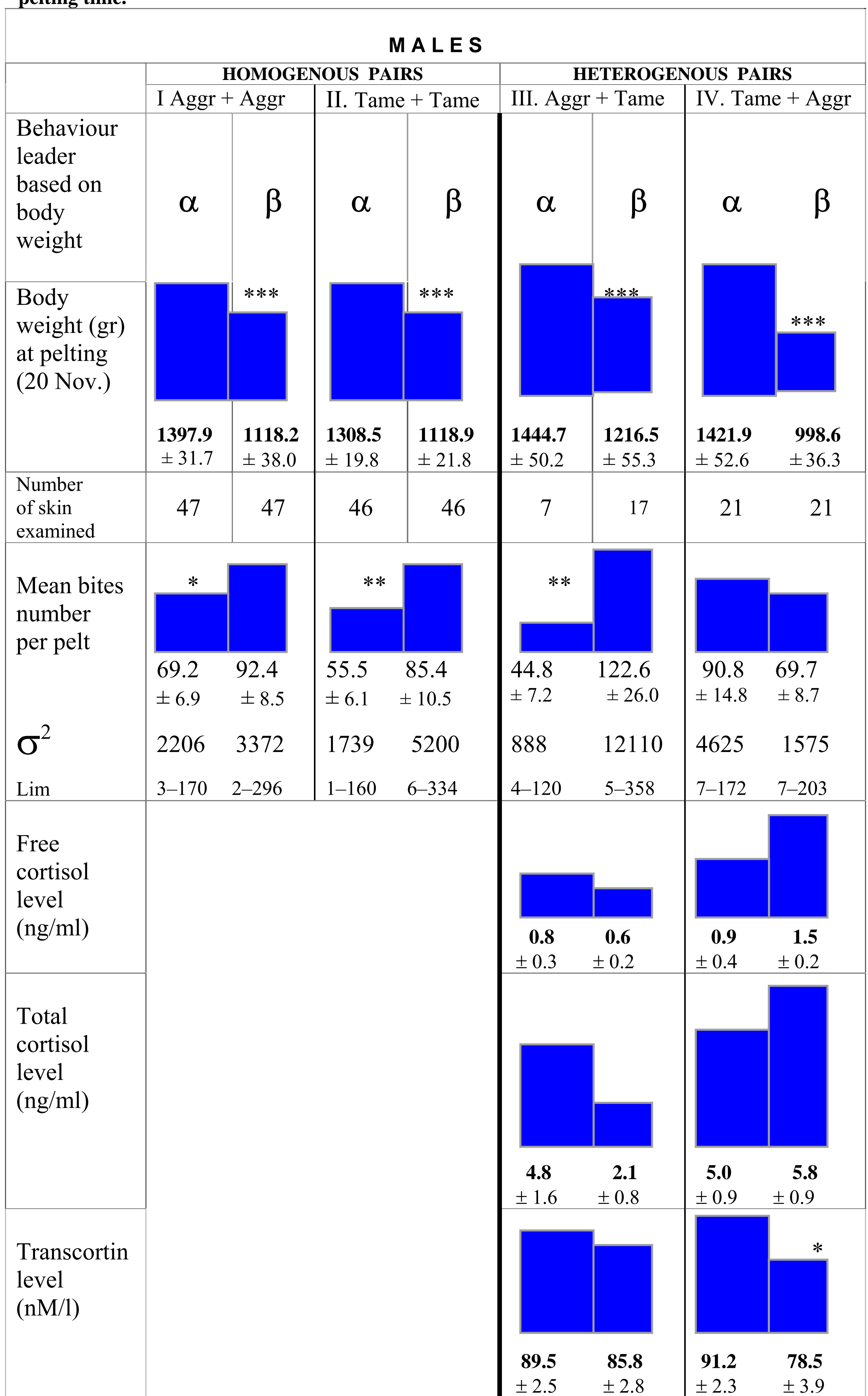
Schribner KA., Walker C-D., Cascio C.S., Dallman M.F. 1991. Chronic streptozotocin diabetes in rats facilitates the acute stress response without altering pituitay or adrenal responsiveness to secretagogues. Endocrinology, 120:99-108.

Tinnikov A.A. 1993. Corticosteroid-binding globuli levels in the rat serum under conditions of starvation and restriction of motions. Horm. Metab. Res. 25, 88-89.

Wade G.N. 1976. Sex hormones, regulatory behaviors, and body weight. Advances in the Study of Behavior, 6, 201-279.

Welch W.L., Welch A.S. 1970. Some aspects of brain biochemistry correlated with general nervous reactivity and aggressiveness. In: C.H.Southwick (Ed.), Animal aggression: Selected readings. New York: Van Nostrand-Reinhold.

Table 1. The results growing of young males different form of behaviour in pairs from weaning to pelting time.



MALES								
	HOMOGENOUS PAIRS				HETEROGENOUS PAIRS			
	I Aggr + Aggr		II. Tame + Tame		III. Aggr + Tame		IV. Tame + Aggr	
Behaviour leader based on body weight	α	β	α	β	α	β	α	β
Body weight (gr) at pelting (20 Nov.)	**1397.9** ± 31.7	*** **1118.2** ± 38.0	**1308.5** ± 19.8	*** **1118.9** ± 21.8	**1444.7** ± 50.2	*** **1216.5** ± 55.3	**1421.9** ± 52.6	*** **998.6** ± 36.3
Number of skin examined	47	47	46	46	7	17	21	21
Mean bites number per pelt	* 69.2 ± 6.9	92.4 ± 8.5	** 55.5 ± 6.1	85.4 ± 10.5	** 44.8 ± 7.2	122.6 ± 26.0	90.8 ± 14.8	69.7 ± 8.7
σ^2	2206	3372	1739	5200	888	12110	4625	1575
Lim	3–170	2–296	1–160	6–334	4–120	5–358	7–172	7–203
Free cortisol level (ng/ml)					**0.8** ± 0.3	**0.6** ± 0.2	**0.9** ± 0.4	**1.5** ± 0.2
Total cortisol level (ng/ml)					**4.8** ± 1.6	**2.1** ± 0.8	**5.0** ± 0.9	**5.8** ± 0.9
Transcortin level (nM/l)					**89.5** ± 2.5	**85.8** ± 2.8	**91.2** ± 2.3	* **78.5** ± 3.9

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ (*Student's "t" test*)

Table 1. The results growing of young females different form of behaviour in pairs from weaning to pelting time.

	FEMALES							
	HOMOGENOUS PAIRS				HETEROGENOUS PAIRS			
	I. Aggr + Aggr		**II.** Tame + Tame		**III.** Aggr + Tame		**IV.** Tame + Aggr	
Behaviour leader based on body weight	α	β	α	β	α	β	α	β
Body weight (gr) at pelting (20 Nov.)	**1164** ± 17.3	*** **1008** ±17.3	**1024** ± 23.7	*** **907** ±18.9	**1127** ± 32.2	*** **925** ±26.6	**1079** ± 24.3	*** **918** ±41.1
Mean bites number per pelt	**30.5** ±5.4	**47.8** ± 8.3	**18.0** ±3.2	**36.2** ± 11.4	*** **17.2** ± 4.5	**43.7** ± 6.7	**28.6** ± 5.4	**35.5** ± 6.1
σ^2	894	2114	294	3646	788	1475	915	1232
Lim	**3 -170**	**2 -296**	**1–160**	**6–334**	**4–120**	**5–358**	**7–172**	**7–203**
Number of skin examined	**31**	**31**	**28**	**28**	**33**	**33**	**29**	**29**

*** $p < 0.001$ (*Student's "t" test*)

I – 13 P

Effect of coat colour mutation in mink on the adrenal cortex function at pelting time in Siberian climate

O.V. Trapezov
Institute Cytology & Genetics, Siberian Department, Academy of Sciences of Russia;
630090, Novosibirsk, RUSSIA. Fax: 7 (3832) 33 12 78; Tel: 7 (3832) 33 05 12.
E-mail: trap@philosophy.nsc.ru (priv), E-mail: trapezov@bionet.nsc.ru (work)

Abstract
Study on the stress reactivity of the organism would be helpful on clarifying the mechanism by which coat colour mutations may affect total viability. To our knowledge, no such study has been performed yet with minks. The aim of the current study was to analyze the effects of the "*hedlund*" and "*aleutian*" mutations, which are common in mink commercial populations, on the adrenocortical function at pelting time (early December) when the temperature in SIBERIA was lowed to – 40ºC at night. Comparison of the obtained data demonstrates that the homozygotes (genotypes *a*/*a*; *h*/*h*; +/+) are quite stressing compared to heterozygotes. The level of 11-oxy in the pooled group of coat colour homozygotes was significantly higher than in heterozygotes (*a*/+; *h*/+). This superiority of minks heterozygous for coat color loci manifest their higher stress tolerance.

Introduction
Perennial questions are related to the influence of coat colour mutations on different physiological and biochemical characteristics, such as reproduction (*Belyaev & Evsikov*, 1967; *Belyaev & Zhelezova*, 1968), growth and development (*Belyaev et al.,* 1977) thermal homeostasis. The questions have been raised with good reason. In fact, development and larger reproduction of a wider range of coat colour hues and patterns are promising trends in effect of coat colour mutations of the adrenal cortex function under environmental conditions in mink.
Study on the stress reactivity of the organism would be helpful on clarifying the mechanism by which coat colour mutations may affect total viability (*Belyaev et al.,* 1977). To our knowledge, no such study has been performed yet with minks. However, from the data in the literature it is known that coat colour genotype in species other than mink may considerably affect the features of emotional behaviour, of the nervous and endocrinological systems, which are the mayor regulators of stress responses, determining both the threshold and intensity of their reactivity.
The aim of the current study was to analyze the effects of the "*hedlund*" and "*aleutian*" mutations, which are common in mink commercial populations, on the adrenocortical function at pelting time (early December) in Siberia.

Materials and Methods
The study was carried out with minks bred at the experimental farm of this Institute. Males of 5 genotypes were studied: Standard (+/+), homozygotes for the recessive mutation "*hedlund*" (*h*/*h*) and "*aleutian*" (*a*/*a*), heterozygotes for these two ones (*h*/+) and (*a*/+). Animals of all groups were maintained in pairs (a mail with female). The experiment was started July, when minks were 3 months old. Animals were fed a standard ration.
The pelting period was at 4-7 December when the temperature outside was lowed to – 40ºC at night. Minks of the genotypic groups were sacrificed on the same day. Promptly after sacrifice adrenals were removed to study their secretion capacity and incubated *in vitro*. The concentration of 11-hydroxycorticosteroids (11-OCS) in the incubation medium was determined fluorimetrically using spectrofluorimeter "Specol".

Results and Discussion
There was a clear-cat trend to higher secretion by homozygotes for the coat colour loci (fig. 1).
This allowed us to divide minks into 2 groups according to the character homo – (genotypes *a*/*a*; *h*/*h*; +/+), and heterozygosity (genotypes *a*/+; *h*/+) and to carry out comparisons. It was found that adrenal secretion in minks homozygous for the examined coat colour loci was significantly higher than in heterozygous.

Fig. 1. Effect of coat color mutations on 11-oxy secretion level by adrenals in conditions of *in vitro* incubation.

♂♂	*Hedlund white h/h*	*Aleutian a/a*	*Standard +/+*	*Standard a/+*	*Standard h/+*	*Homo-zygotes: a/a; h/h; +/+*	*Hetero-zygotes: a/+; h/+*
Secretion *in vitro* (mg/g/h) (11.0 – 4.0)							*
Number of animals	8	11	9	8	9	28	17
The level of 11-oxy	10.7 ± 2.0	10.3 ± 1.6	10.3 ± 1.5	7.8 ± 1.3	7.4 ± 1.2	10.4 ± 0.9	7.6 ± 0.9

**– significant*

To understand this, it is well to recall that paired cage maintenance of young minks in November-December, when temperature in Siberia drops to – 40ºC in the night time, was very demanding. This caused mobilization of the adaptive mechanisms, including stress systems. The minks homozygous for the standard (+/+) and mutant alleles (*a*/*a*; *h*/*h*) were tensed at a higher level than the heterozygotes (*a*/+; *h*/+). That animals are stressed at this time of the year when placed in pairs.

Comparison of the obtained data demonstrates that in the homozygote (genotypes *a*/*a*; *h*/*h*; +/+) when winter temperature fall to – 40ºC are quite stressing compared to heterozygotes. The level of 11-oxy in the pooled group of coat colour homozygotes was significantly higher than in heterozygotes (*a*/+; *h*/+).

Concluding, it is noteworthy that here we are dealing with a manifestation of heterosis due to a broad pleiotropic effect of alleles controlling the biosynthesis and distribution of pigments. It appears likely that a most important genetic-physiological mechanism providing heterosis is involvement of genes for the neuroendocrine and adrenocortical system provoking adaptation to unfavorable factors in the pleiotropic effects.

This superiority of minks heterozygous for coat color loci manifest their higher stress tolerance.

References

Belyaev D.K., Evsikov V.I. 1967. The genetics of animals reproductivity. Report I. The influence of colour phases on the minks reproductivity. Genetika. № 2. P. 21-33 (in Russian).

Belyaev D.K., Zhelezova A.I. 1968. Some physiological particularity of mutant minks reproductivity. Genetika. V. 4. № 1. P. 45-57 (in Russian).

Belyaev D.K., Schuler A., Borodin P.M. 1977. The problems of stress genetics. Report III. The differentiation role of stress on the mouse reproductivity in deferens stocks. Genetika. V. 13. № 1. P. 52-58 (in Russian).

I – 14 P

Young nutria behaviour in runs of different types

O.Yu. Bespyatykh
Russian Research Institute of Game Management and Fur Farming,
79 Engels Street, Kirov, 610000, Russia,
e-mail: mink@mink.kirov.ru

Abstract
The comfort of runs of different types for nutria keeping was estimated with the method of ethological observations. The behaviour of nutria females and males at the age of six months was studied. Animals were visually watched during 24-hour periods. 36 elements of behaviour were totally registered, 13 of them being the main ones. Some runs have a typical wire mesh floor. In other runs metal shelves that occupied 1/3 of a floor area were set up. Young nutria used shelves not only for feeding, but also for different elements of an intensive and local activity, for rest and sleep. As compared with a wire mesh floor, young animals rested on shelves for a longer time, the length of comfort behaviour increased. Thus, shelves in a run raised the comfort of nutria keeping.

Introduction
Behaviour is the most effective mechanism of animals' adaptation to living conditions. Any change in environment resulted in the change of behaviour that is the first and easily recognized response of animals. When studying behaviour response in usual and changed conditions it is possible to make conclusion about the trends (favourable or unfavourable) in which the environmental conditions change (Kavalcikova & Kovalcik, 1974).
When carrying out farm breeding, animals' behaviour may be used to estimate the ecological effect of different zootechnical measures and to improve conditions of animal keeping (Korytin & Zabotsckikh, 1983; Kholeva, 1997). The principle of creating comfort conditions of keeping and welfare of animals (absence of disturbances of behaviour and general status of animals) was entered in the Recommendations concerning fur animals – T-AP (96) 19 (1999), adopted by Council of Europe and directed to agricultural animal conservation.
Earlier we designed a shelf (a feeding table) to decrease the losses of feed for nutria. It was a metal plate with a plane surface and low sides. A shelf decreased the losses of feed for nutria more than three times, increased the growth rate of the young by 9-12 % and its survival by 3-9 % (Bespyatykh & Plotnikov, 2000, 2002).
We studied the effect of a shelf placed in a run on the behaviour of the nutria's young. When analyzing animals' behaviour a comfort level of runs of a different type was estimated.

Materials and Methods
Investigations were carried out at the nutria nursery of the fur-bearing animal farm "Pushnina" (Kirov region). Animals were kept indoors with a regulated microclimate in runs without a house and a pool.
Behaviour of females and males of the nutria's young was studied in typical and improved runs. A typical run was made of a metal wire mesh measuring 0.8x0.6x0.35 m with holes 25x25 mm in diameter. Along a short side of a run there was a feed tray, at an opposite side, in the corner there was a drinking bowl. Water was poured into a bowl from 8 to 17 o'clock. Granulated mixed feed (granules' diameter – 10 mm) was given once a 24-hour period in the morning. An improved run had the same size as a typical one. But in addition to a feed tray a metal shelf was set up. It had a plane surface 0.6x0.22 m in size and sides 3 cm in height. The shelf occupied one third of a floor area of a run.
Nutrias' behaviour was studied through 24-hour visual observations of animals. The elements of behaviour were noted in 15-minute intervals (Scholz et al., 1964; Kovalcikova & Kovalcik, 1974). In a dark period of a day observations were carried out with little electric lighting. In all, 36 elements of behaviour of young animals were noted.
Experimental groups were formed of young nutrias at the age of 6 months. Animals of the first group were kept in a typical wire mesh run (males n=9, females n=12), animals of the second group – in a run with a shelf (males n=9, females n=12).

Results

During ethological observations 36 elements of behaviour were taken into account, 13 of them being the main ones.

In nutria males kept in the runs with shelves as compared with the males in typical runs the ratio of the categories of activity and rest in their behaviour practically did not change. Thus, in typical runs an intensive activity in males was registered during 0.84 hour, a local activity – 14.67 hours, sleep – 7.97 hours in a 24-hour period. In the runs with shelves those indices were 0.80, 14.45, 8.25 hours in a 24-hour period, correspondingly.

But in those categories the duration of the elements of behaviour changed (Table 1). In typical runs males sat on a wire mesh floor by 41.2 %, slept in a sitting position by 15.8 % and drank water by 34.1 % longer as compared with the males in the runs with a shelf. In typical runs males lay on a side by 21.8 %, lay on a belly by 69.1 % (P<0.05), slept on a side by 18.9 %, slept on a belly by 66.0 % and ate feed by 15.6 % less than males in the runs with a shelf. Other elements of behaviour of the nutria’s young had no significant differences between groups.

To reveal preferences in the young (young animals prefered to be on a shelf not only to feed, but also to rest) we equated the area of a wire mesh floor in a run to the area of a shelf.

On a wire mesh floor of a typical run we noted an intensive activity in animals during 0.24 hour, a local activity – during 3.29 hours, sleep – during 1.11 hours per a 24-hour period. On a wire mesh floor of a run with a shelf those indices made up 0.28, 4.29, 2.28 hours per a 24-hour period, on a shelf – 0.1, 6.24, 5.45 hours per a 24-hour period, correspondingly.

In that case the elements of behaviour had the following indices (Table 2). On a wire mesh floor of a typical run males moved 1.5 times as many and were in contact with a neighbour 5 times more frequently than males on a shelf. On a wire mesh floor of a typical run animals sat 1.2 times, lay on a belly 6.5 times (P<0.05), slept in a sitting position by 1.8 times, slept on a side by 3 times, slept on a belly 9.2 times, pawed their hair cover by 1.4 times and ate feed by 3.6 times (P<0.01) as many in comparison with the animals on a shelf. Other elements of behaviour of the nutria,s young did not have any significant differences between groups.

Females of nutria had a similar behaviour in typical runs and in runs with shelves as males.

Table 1Behaviour elements in nutria males in runs of different type during a 24-hour period, hour

Behaviour elements	Control (n=9)	Experiment (n=9)		
		Totally	On wire net floor	On shelf
Laying on side	1.97 ± 0.24	2.52 ± 0.89	1.99 ± 0.58	0.53 ± 0.41 $*^{01}$
Sleeping on side	2.74 ± 0.50	3.38 ± 1.34	1.01 ± 0.55 $*^{01}$	2.35 ± 1.08
Laying on belly	0.34 ± 0.17	1.10 ± 0.22 $*^{05}$	0.46 ± 0.05	0.65 ± 0.19
Sleeping on belly	0.17 ± 0.12	0.50 ± 0.29	0.05 ± 0.05	0.46 ± 0.31
Moving	0.53 ± 0.02	0.70 ± 0.38	0.60 ± 0.46	0.10 ± 0.10 $*^{001}$
Climbing on wire mesh	0.31 ± 0.17	0.10 ± 0.10	0.10 ± 0.10	0
Contact with neighbours	0.36 ± 0.24	0.36 ± 0.10	0.34 ± 0.07	0.02 ± 0.02 $^{+001}$
Feeding	2.18 ± 0.14	2.52 ± 0.38	0.29 ± 0.05 $*^{001}$	2.23 ± 0.41 $^{+001}$
Drinking of water	1.32 ± 0.07	0.87 ± 0.17 $*^{05}$	0.86 ± 0.17 $*^{05}$	-
Pawing of hair cover	1.66 ± 0.34	2.16 ± 0.12	1.49 ± 0.17	0.67 ± 0.05 $*^{05,\ +001}$
Sitting	6.41 ± 0.77	4.54 ± 1.18	2.40 ± 0.48 $*^{001}$	2.14 ± 0.74 $*^{01}$
Slipping in sitting position	5.06 ± 1.27	4.37 ± 1.70	1.73 ± 1.01	2.64 ± 0.86
Pulling of wire mesh with teeth	0.43 ± 0.02	0.38 ± 0.02	0.38 ± 0.02	0

$*^{01}$ *- differences are reliable with control, P<0.01*

$*^{05}$ *- differences are reliable with control, P<0.01*

$*^{001}$ *- differences are reliable with control, P<0.001*

$^{+001}$ *- differences are reliable with wire mesh floor, P<0.001*

Table 2 Behaviour elements in nutria males on equaled areas of shelf and wire net floor of typical runs during a 24-hour period, hour

Behaviour elements	Control (n=9)	Experiment (n=9)	
		On wire net floor	On a shelf
Laying on side	0.56±0.07	0.80±0.23	0.53±0.41
Sleeping on side	0.78±0.14	0.40±0.22	2.35±1.08
Laying on belly	0.10±0.05	0.18±0.02	0.65±0.19 $*^{05, +05}$
Sleeping on belly	0.05±0.03	0.02±0.02	0.46±0.31
Moving	0.15±0.01	0.24±0.18	0.10±0.10
Climbing on wire mesh	0.09±0.05	0.04±0.04	0
Contact with neighbours	0.10±0.07	0.14±0.03	0.02±0.02 $^{+01}$
Feeding	0.62±0.04	0.12±0.02 $*^{001}$	2.23±0.41 $*^{01, +001}$
Drinking of water	0.38±0.02	0.34±0.07	-
Pawing of hair cover	0.47±0.10	0.60±0.07	0.67±0.05
Sitting	1.83±0.22	0.96±0.19 $*^{01}$	2.14±0.74
Slipping in sitting position	1.45±0.36	0.69±0.40	2.64±0.86
Pulling of wire mesh with teeth	0.13±0.01	0.15±0.01	0

$*^{01}$ *- differences are reliable with control, P<0.01*
$*^{05}$ *- differences are reliable with control, P<0.01*
$*^{001}$ *- differences are reliable with control, P<0.001*
$^{+01}$ *- differences are reliable with wire mesh floor, P<0.01*
$^{+05}$ *- differences are reliable with wire mesh floor, P<0.05*
$^{+001}$ *- differences are reliable with wire mesh floor, P<0.001*

Discussion

After setting up a shelf in a run the elements of behaviour in males, i.e. of rest– periods of laying on a side and a belly, sleeping on a side and a belly became longer. It took place due to the decrease of the duration of sitting, sleeping in a sitting position and drinking water. The increase of the time of feeding was explained by the fact that a shelf favoured the decrease of feed losses (Bespyatykh & Plotnikov, 2000, 2002). In a feed tray more feed remained, and animals ate it more quietly, slowly and without fighting.

The equaling of a wire mesh floor area of a typical run to an area of a shelf showed that on a wire mesh floor of a run the young preferred to move along a run, to climb a wire mesh, to pull it with teeth and to contact with neighbours through it. Thus, on a wire mesh floor of a run animals showed the elements of an intensive activity. On a shelf males of nutria preferred to sit, to lie, to sleep and to paw their hair cover. On a shelf the young showed the elements of a local activity.

The increase of a local activity is characteristic of the level of animals’ adaptation to living conditions and of the level of an ecological comfort and optimum (Korytin &0 Zabolotskikh, 1983). In our case a local activity of nutria is greater on a shelf than on a wire mesh floor of a run. It proved that animals preferred to be not on a wire mesh floor, but on a shelf and not only for feeding.

Therefore, a shelf corresponds to biological peculiarities of nutria and favours the increase of the level of runs’ comfort.

Conclusion

Setting up of a shelf in nutria runs increases the level of comfort conditions of keeping the young. It resulted in changes of animals’ behaviour. The duration of a local activity increases, i.e. of the elements of comfort behaviour (lying, pawing their hair cover). The length of an intensive activity decreases.

Comfort conditions of nutria keeping will give an opportunity to obtain from animals the greatest total volume of skin and meat output.

References

Bespyatykh, O.Yu, Plotnikov, I.A. 2000. Decrease of food losses in feeding nutria. Scientfur. 24. 4:72-73.

Bespyatykh, O.Yu.,Plotnikov, I.A. 2002. Decrease of feed losses in nutria. Pages 539-541 *in* Recent problems of nature use, game biology and fur farming: Proceedings of the International Scientific and Practical Conference. Kirov (in Rus.).

Kholeva, P. 1997. Behaviour of nutria of different age. Krolikovodstvo I Zverovodstvo. 5:14 (in Rus.).

Korytin, S.A., Zabolotskih, Yu. S. 1983. Comparative characteristic of motion activity of canidae. Volume 2, Pages 204-206 *in* Behaviour of animals in community: Proceedings of III All-Union Conference on Animal Behaviour. Moscow (in Rus.).

Kovalcikova, M., Kovalcik, K. 1974. Adaptacia a stress v chove hospodarskych zvierat. Bratislava (in Czech).

Recommendations concerning fur animals – T-AP (96) 19. 1999.

Scholz, K., Himmel U., Lips, C. 1964. Problematik, Methodic und Ergebnisse von unterschungen zum Verhalten der Rinder und Schweine in Grossbestanden. Arch. Tierzucht. 7.1:3-20 (in Germ.).

SCIENTIFUR
ISSN 0105-2403
Vol. 28, No. 3

Proceedings of the VIII International Scientific Congress in Fur Animal Production

II: Health

Edited by:

Dr. Bert Urlings
Prof. Dr. Berry Spruijt
Dr. Marko Ruis
Ing. Louise Boekhorst

II – 1 RP

Management of health in mink
A HACCP plan for energy allowance during winter and gestation in order to control sticky kits

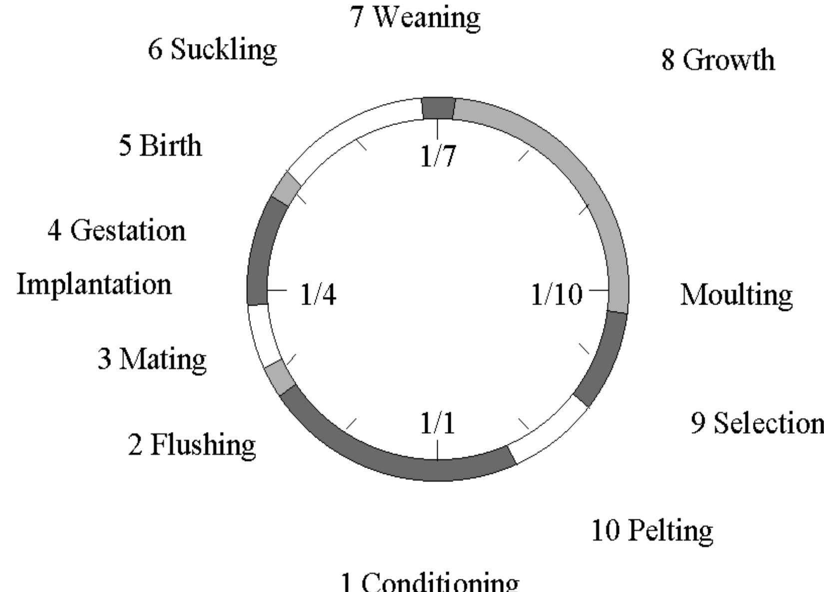
S.H. Møller
Department of Animal Health and Welfare, Danish Institute of Agricultural Sciences, Research Centre Foulum, Denmark.
E-mail steenh.moller@agrsci.dk

Abstract
Mink production is characterised by a strict annual production cycle in which all animals are naturally synchronous and the number of animals in a given mink production period is high. Each age group presents its own health problems and the whole herd is at risk to period-specific hazards. Due to a significant time lag in the feedback-loop of mink management, health management should focus strongly on preventive measures. The HACCP principles offer a systematic approach to the development of preventive measures aimed at annually recurrent health problems. The hazard of inadequate energy supply during the winter and during pregnancy has been identified as a risk factor for pre-weaning diarrhoea. The severity and seriousness of the consequences of this hazard has been analysed at farm level based on production data from 5 years (1994-1998) from a total of 125 farms in Denmark. Two hazards for pre-weaning diarrhoea at farm level have been identified: 1). Severely restricted feeding during the winter followed by a high energy allowance during the flushing period. A difference between the Flushing and Conditioning periods of 90 kcal/female/day at farm level increases the risk (OR = 3.03; p=0.01). 2). A high energy allowance during the implantation period and the first part of the gestation period followed by a drastic decrease in the later part of the gestation period. By each kcal/female/day in difference between the Implantation and Prenatal two periods the risk of pre-weaning diarrhoea at farm level increases by OR=1,013 (p<0,05). A difference between the two periods of 50 kcal/female/day at farm level increases the risk by OR = 1,91. Following the hazard evaluation the average feed allowance in kcal/female/day can be identified as Critical Control Point for both hazards. Critical limits may be established as the average ± one unit of STD for the group of farms without pre-weaning diarrhoea or ± 20 kcal relative to an estimated "need" of 210 kcal/female/day.

Introduction
The general health status of the farm mink is good and the mortality is low apart from the first days after birth (Dietz et al., 2000; Durrant, 2000; Rattenborg et al. 1999; Schneider & Hunter 1993a; 1993b) while use of antimicrobials varies considerably between farms and production periods (Chriél & Dietz, 2000).
The management of health in mink production is to a large extent defined by the same factors that in general characterise management of synchronised production systems: a time lag in the management feed back cycle.

Seasonal synchrony
Mink production is characterised by a strict annual production cycle in which all animals are naturally synchronous. In the northern hemisphere, all female mink may be successfully mated within 3 weeks in March, litters are delivered within three weeks around 1 May and all animals are pelt prime in November. Mink kits join the annual cycle already during the first year, as they are synchronous in terms of body weight and pelt moulting 4-6 months after birth. As indicated in Fig 1, mink production can be divided into ten distinct seasonal production periods differing in terms of management, length, labour intensity, number, age and sex of the mink, season, mortality and risk of disease (Møller, 1999; Møller & Sørensen, 2004).

Fig. 1. Synchronous periods in the seasonal mink production cycle. Numbered periods indicate a need for special management routines. The centre dial indicates the respective calendar dates (from Møller, 1999; Møller & Sørensen, 2004).



Compared to continuous production systems, the number of animals in a given mink production period is high. Each age group presents its own health and welfare problems and different risk factors for these problems are often limited to a narrow period of time once a year (Møller et al., 2003). The number of mink affected by management procedures is high and the whole herd is at risk to period-specific hazards. Furthermore, many farms are subject to similar risk factors due to very uniform housing and management systems, feed composition, etc.

Management

Health management in a mink farm can be described as a cybernetic system (Sørensen & Kristensen, 1992; Møller & Sørensen, 2004). In this context the farm is organised as a production system defined by the animals, buildings, machines, land and labour, and a management system defined by feedback of information performed by the farmer (Fig. 2). It is an open system, as it produces animal products and by-products by use of controllable and uncontrollable inputs (e.g. climate, feed quality and ingredients, infectious diseases). By regulating the controllable factors the farmer tries to maintain the production in harmony with the goal while adjustments are needed, when uncontrollable factors induce deviations from the goal. The interaction between the production system and the management system is illustrated in Fig. 2. Management is seen as a chronological series of: 1. Measurements of the production system's behaviour. 2. Comparison with a goal or a plan. 3. Adjustment in controllable factors

At the operational level clinical disease should be treated correctly and as fast as possible. However, the effect of management, infectious organisms or other health hazards in one production period is often seen in consecutive production periods. Therefore, corrective actions towards an observed effect must often be postponed until the relevant production period next year because the relevant adjustment is aimed at a previous production period (Møller, 1999; Møller & Sørensen, 2004). Due to this significant time lag in the completion of the feedback-loop, the management of health in mink at the tactical level should focus strongly on preventive measures. Hence there is a need for a systematic approach to the development of preventive measures aimed at annually recurrent health and welfare problems. Such an approach is offered by the HACCP principles.

Fig.2. Mink production as a cybernetic system exemplified by health management (modified from Sørensen & Kristensen, 1992; Møller & Sørensen, 2004).

The management system

The mink farmer compares the health status with the goals. A deviation from the goals may initiate an adjustment.

Adjustment in controllable factors

Measurement of system's behaviour

The production system

Buildings

Machines

Labour

Male mink

Female mink

Controllable factors

(e.g. vaccinations, feeding strategy, water supply)

Uncontrollable factors

(e.g. infectious agents, feed quality, temperature, humidity)

Healthy mink
Diseased mink
Virus, Bacteria

Manure
Wastewater

In this paper, preventive health management focusing on the HACCP principles is shortly discussed and the process is exemplified by a hazard for the pre-weaning diarrhoea syndrome often termed 'sticky', 'greasy' or 'wet' kits in mink.

Preventive health management and the application of HACCP

Vaccination

Classical preventive measures like vaccinations are widely used in mink production and commercial vaccines are available against viral diseases like distemper and virus enteritis and against bacterial diseases like botulism and pseudomonas infection.

HACCP

The principles of Hazard Analysis and Critical Control Point (HACCP) are developed for assuring food safety from harvest to consumption: "Preventing problems from occurring is the paramount goal underlying any HACCP system. Seven basic principles are employed in the development of HACCP plans that meet the stated goal. These principles include hazard analysis, CCP identification, establishing critical limits, monitoring procedures, corrective actions, verification procedures, and record-keeping and documentation. Under such systems, if a deviation occurs indicating that control has been lost, the deviation is detected and appropriate steps are taken to re-establish control in a timely manner to assure that potentially hazardous products do not reach the consumer" (NACMCF, 1998).

Urlings & Koenen (2000) outlined a health management system for use in mink farms by a mink veterinary practice including the HACCP principles. According to these principles known hazards are analysed and significant hazards are, if possible, controlled at critical points in due time before they may cause disease. As outlined above, preventive measures like HACCP are well suited in mink production, due to the time lag in the health management feed back cycle.

One problem using HACCP in mink production is to identify hazards that will cause disease or threaten the health of the mink if they are not controlled and to evaluate which of these hazards should be addressed in the HACCP plan. This involves considerations of severity and likely occurrence for which the scientific knowledge is often insufficient. Last but not least, it must be possible to specify CCPs that may effectively prevent or reduce the hazard threatening the health of the mink if the hazard is controlled at this point.

The following example shows the process of recognising a hazard, analysing the risk, calculating odds ratios and defining safe limits wherein the CCP should be maintained in order not to become a hazard.

A hazard for pre-weaning diarrhoea

Pre-weaning diarrhoea syndrome, also known as 'sticky' or 'greasy' or 'wet' kits have been a health problem in Danish mink production for at least 50 years (Svennekjær, 1954; Clausen & Dietz, 2004). The syndrome involves diarrhoea as well as secretion from cervical apocrine glands (Englund et al., 2002). Numerous risk factors have been suggested over the years of which few have been supported by biological arguments end even fewer by data.

Hazard analysis

Hazard identification

A number of risk factors e.g. 1-year females, large litter size, late date of birth and inadequate energy supply during the winter and late pregnancy, low body weight and the presence of astrovirus, coccoid bacteria and calicivirus gains support from an increasing number of studies (Olesen & Clausen, 1990; Chriél, 1994; 1997; Hillemann, 1996; Møller & Chriél, 2000; 2001, Englund et al. 2002). Risk factors like 1-year females, large litter size and late date of birth are part of the mink production and thus not hazards that can be prevented at critical control points. Hazards like inadequate energy supply during the winter and late pregnancy, low body weight and the presence of astrovirus, coccoid bacteria and calicivirus can potentially be prevented as risk factors and are thus candidates as hazards for which CCPs can be identified. The hazard of inadequate energy supply resulting in low body weight or excessive weight loss as a risk factor for pre-weaning diarrhoea has been reported by (Olesen & Clausen, 1990; Møller, 1994; Møller & Chriél, 2000). Later on a correlation at farm level between feeding strategy during the winter and during pregnancy was realised and the importance of inadequate energy supply during the gestation period as a risk factor was investigated and reported (Chriél, 1994; 1997; Møller & Chriél, 2000; 2001).

Hazard evaluation

Although the hazard and its occurrence in practice was identified, the severity and seriousness of the consequences could not be properly evaluated. E.g. the results by Møller & Chriel (2000) were based on very detailed registrations on 6 farms which was too few for providing conclusive evidence regarding risk factors and odds ratios relative to other relevant factors. Therefore an epidemiological analysis at farm level has been performed based on production data from 5 years (1994-1998) from a total of 125 farms receiving feed from one of the largest mink feed producers in Denmark. Reproduction and herd size (number of female breeders on the farm) data were collected from the breeding system DanMink. Data on feed delivered to the farm as well as the number of mink on the farm was collected from the feed plant and the average feed allowance was calculated on a weekly basis for weeks 1 to 18 (from the beginning of January to the beginning of May). The occurrence and incidence of the pre-weaning diarrhoea syndrome was collected by an annual questionnaire. Four characteristic feeding periods were defined (weeks refer to the time period of the year, e.g. week 1 is the first week in January).

1. Conditioning – week 1-8.
2. Flushing – week 9-12.
3. Implantation - week 13-15.
4. Prenatal – week 16-18.

In each period the average feed allowance per mink was calculated for each farm and year. As the energy requirement of the mink may vary between farms and years, the difference in kcal/female/day between Flushing and Conditioning and between Implantation and Prenatal was calculated for each farm and year and used in the calculations (Table 1). For almost all factors, farm was the level of observation, but the colour types (Brown, Black and Others) within each farm and year were also registered. As the incidence of sticky kits was not registered per colour type, this factor was not included in the analysis. Furthermore, colour type i.e. the percentage of Other mink than Brown or Black was confounded with herd size. Herd size was included in the model as the chance to find infected litters increases with herd size irrespective of other factors.

The effect of feeding strategy on the risk of sticky kits on the farm was examined. The following factors were included in the model:

- The difference in kcal/female/day between Flushing and Conditioning
- The difference in kcal/female/day between Implantation and Prenatal
- Herd size
- Farm
- Year

Table 1. Average energy allowance per mink in kcal/female/day in the Conditioning, Flushing, Implantation and Prenatal periods, as well as the differences Flushing - Conditioning and Implantation - Prenatal.

	N	Conditioning	Flushing	Diff Fl-Co	Implantation	Prenatal	Diff Im-Pr
No sticky kits	160	198±18	253±23	55±26	228±23	215±20	13±26
Sticky kits	132	194±17	256±24	63±30	232±19	208±17	24±21

The risk factors were tested in a univariable logistic regression model with Farm as repeated effect between years. An autoregressive correlation structure was chosen because management of subsequent years seems more likely to be related.
Complete data from 125 breeders with 1-5 years of observations were analyzed. From 47 farms only one year with complete data was available, while only 9 farms had complete data from all 5 years.
The analysis showed that large difference in feed allowance between the Flushing and Conditioning periods and between the Implantation and Prenatal periods as well as herd size significantly increases the risk of pre-weaning diarrhoea at farm level. A difference between the Flushing and Conditioning periods of 90 kcal/female/day at farm level increases the risk (OR = 3.03; p=0.01). By each kcal/female/day in difference between the Implantation and Prenatal two periods the risk of pre-weaning diarrhoea at farm level increases by OR=1,013 (p<0,05). A difference between the two periods of 50 kcal/female/day at farm level increases the risk by OR = 1,91.
In other words, two hazards for pre-weaning diarrhoea at farm level have been identified: 1). Severely restricted feeding during the winter making room for a high energy allowance during the flushing and mating period. This result is in accordance with previous results (Olesen & Clausen, 1990; Møller, 1994; Møller & Chriél, 2000). 2). A high energy allowance during the implantation period and the first part of the gestation period followed by a drastic decrease in the later part of the gestation period. This confirms and quantifies previous results by Chriél (1994; 1997) and Møller & Chriél (2000; 2001).

CCP identification
“A critical control point is defined as a step at which control can be applied and is essential to prevent or eliminate a (food safety) hazard or reduce it to an acceptable level.” (NACMFC, 1998). Following the hazard evaluation the average feed allowance in kcal/female/day can be identified as Critical Control Point for both hazards. The feed allowance may be calculated based on the amount of feed delivered from the feed kitchen and the number of mink on the farm, or by the energy content of the feed and the feed allowance fed to the mink. Another potential Critical Control Point could be the average weight or the weight change during the period

Establishing critical limits
“A critical limit is a maximum and/or minimum value to which a biological, chemical or physical parameter must be controlled at a CCP to prevent, eliminate or reduce to an acceptable level the occurrence of a food safety hazard. A critical limit is used to distinguish between safe and unsafe operating conditions at a CCP. Critical limits should not be confused with operational limits which are established for reasons other than food safety.” (NACMFC, 1998). An acceptable level of pre-weaning diarrhoea other than 0 cases is difficult to establish. For both identified hazards an upper and lower critical limit for the CCP “feed allowance” should be given. Such limits can not be deducted directly from the data analysis performed hitherto, but upper and lower limits could be given relative to the average feed allowance from January to April or relative to the need of the female mink. While the energy “need” of female mink has not been established, an average voluntary feed intake during the entire period of 200 kcal ME/day has been reported by Hansen et al., (1991). Due to large variation in management, nest box insulation, body weight and activity pattern of the mink the feed allowance and probably also the energy need varies between years, farms and even between colour types and individual mink within each farm. Somehow, the safe limits should be able to reflect these differences. However, until such methods are developed, a pragmatic way to establish critical limits could be the average ± one unit of STD (standard deviation) for the group of farms without pre-weaning diarrhoea or relative to an estimated “need” of 210 kcal/female/day for todays mink as suggested by Møller & Chriel (2000) (Table 2).

Table 2. Critical limits for average energy allowance per mink in kcal/female/day based on observed means ± 1 unit of STD or relative to en estimated "need". Limits defined for the Conditioning, Flushing, Implantation and Prenatal periods, as well as for the differences Flushing - Conditioning and Implantation – Prenatal.

	Conditioning (Co)	Flushing (Fl)	Difference Fl-Co	Implantation (Im)	Prenatal (Pr)	Difference Im-Pr
Upper limit (+1STD)	216	276	81	251	235	39
Lower limit (-1STD)	180	230	29	205	195	0
Upper limit ("need")	-	-	-	230	230	(40)
Lower limit ("need")	190	190	-	190	190	-

As insufficient supply of energy and nutrients are the probable biological factor behind the risk for pre-weaning diarrhoea there is no doubt that lower limits are needed in the conditioning and prenatal periods. It is however, open for debate whether upper limits are needed for the flushing and implantation periods. If so, they are merely an indirect limit to support the control of the lower limit in the prenatal period.

Discussion

The problem of excessive weight loss/low body weight due to restricted feeding /inadequate energy supply follows from the farmers wish to utilize the effect of flushing. The effect of flushing has been documented when females in low-to-moderate body condition are fed restricted for a couple of weeks, followed by *ad libitum* feeding from 5 days before the onset of mating until second mating (Tauson, 1993). In order to maximize their litter size most mink farmers have adapted this feeding strategy. However, many farmers feed more restricted during the winter than indicated by experimental results because the weight of new dams selected for breeding among kits in pelting condition is increasing. In practice weight reductions in the period from November to February of up to 40 % have been observed even though no effect of a more severe weight reduction than the 15-20% that will often follow a correct flushing have been documented (Møller, 2000). A report by Børsting & Hedegaard (1998) indicating a positive effect on litter size of high energy allowance during the implantation period in late March - beginning of April has inspired many farmers to continue the *ad libitum* feeding of the mated females in this period. In order to prevent maternal dystocia these farmers often drastically reduce the feed allowance prior to delivery in late April. However, growth of foetal (Tauson et al., 1992) and mammary-gland (Møller, 1996) tissues is intense during the later part of the gestation period in April and a low energy allowance has been found to decrease the females' lactation capacity in the nursing period (Møller, 1994; Brzozowski & Møller, 1996) and to reduce the amount of mammary gland tissue 6 weeks post partum (Møller & Sørensen, 1999).

Although safe limits between 190 and 230 kcal/female/day for energy allowance during gestation has been suggested in Denmark (Møller & Chriel, 2000) this CCP has not been widely accepted or incorporated in health management at Danish mink farms. This is probably due to the fact that the exact risk for pre-weaning diarrhoea was not documented and safe limits were somewhat arbitrarily set. Another reason may be that the upper limit which is the most opposed in the Danish debate is not a hazard in itself, but merely induces the hazard of insufficient energy allowance in the later part of gestation. Therefore, the HACCP process and quantification of the risk and critical limits may increase the acceptance.

As the effect of energy allowance during implantation on litter size is inconclusive (Lund, 1992; Kemp et al., 1993; Børsting & Hedegaard, 1998) an analysis of the effect of energy allowance during flushing as well as during implantation on litter size based on a sufficient data material is needed in order to clarify the farmers motive for hazardous feeding during the gestation period.

Literature

Brzozowski, M. & Møller S.H. 1996. Development of the female mink mammary gland during gestation and lactation in relation to feeding intensity. Progress in fur animal science, Proceedings from VIth International Scientific Congress in Fur Animal Production, August 21-23, Warsaw, Poland. Polish Society of Animal Production, Applied Science Reports 27, 103-113.

Børsting, E.& Hedegård, E. 1998. Fodring i drægtighedsperioden. Resultater fra farmpanel ved Sole Minkfoder. Dansk

Pelsdyravl 61. 120-121.

Clausen, T.N. & H.H.Dietz. 2004. Wet kits in mink. A review. VIII International Scientific Congress in Fur Animal Production, Holland, Sept. 2004.

Chriél, M. 1994. Wet mink kits, an Epidemiological Investigation of Risk Factors. In XVII Nordic Veterinary Congress, 26-29 July, Reykjavik, Island.

Chriél, M. 1997. Lad minktæverne selv bestemme! Resultater fra den epidemiologiske undersøgelse af fedtede hvalpe i 1996. Dansk Pelsdyravl, 60, 196-198.

Chriél, M. & H.H. Dietz. 2000. Medication in Danish mink farms. Proc. VIIth Int. Sci. Congress in Fur Animal Prod. Scientifur 24, 4-VI, 32-36.

Dietz, H.H., Andersen, T.H. & Chriél, M. 2000. Health surveillance in Danish mink farms: A prospective study. Proceedings from VIIth International Scientific Congress in Fur Animal Production, September 13-15, Kastoria, Greece. Scientifur. Vol 24, 4-VI, 13-17.

Durrant, G.R. 2000. Mortality in ranch raised mink: A year in review. Proceedings from VIIth International Scientific Congress in Fur Animal Production, September 13-15, Kastoria, Greece. Scientifur Vol 24, 4-VI, 11-13.

Englund, L., Chriél, M., Dietz, H. H. & Hedlund, K. O. 2002. Astrovirus epidemiologically linked to pre-weaning diarrhoea in mink. Veterinary Microbiology. 85, 1-11.

Hillemann, G. 1997. Fedtede hvalpe. Et kort sammendrag af spørgerskemaundersøgelserne gennemført af Nordjysk Minkfoder A/S i årene 1994, 1995 og 1996. (Sticky kits. A short review of questionaires performed at Nordjysk Minkfoder A/S in 1994, 1995 and 1996). Dansk Pelsdyravlerforening, Faglig Årsberetning 1996. 225-226.

Kemp, B.; Martens, R.P.C.H.; Hazeleger, W.; Soede, N.M. & Noordhuisen, J.P.T.M. 1993. The effect of different feeding levels during pregnancy on subsequent breeding results in mink (Mustels vison). Journal of Animal Physiolgy and Animal Nutrition. 69, 115-119.

Lund, R.S. 1992. Foderstyrke. Forsøg vedr. foderstyrkens indflydelse i implantationsperioden på hvalperesultat og dieperiodens forløb. (Feed allowance. Investigations regarding the effect of feed allowance during the implantation period on litter size and the performance in the lactation period) Dansk Pelsdyravl, 55. p. 143.

Møller, S.H. 1994. Restriktiv fodring af drægtige minktæver i perioden op til fødsel. (Restricted feeding of pregnant females in the pre natal period). NJF seminar No. 253. 28.-30. September, Rebild, Danmark, 9 pp.

Møller S.H. 1996. Development of the mammary glands in female mink from weaning through first lactation. Progress in fur animal science, Proceedings from VIth International Scientific Congress in Fur Animal Production, August 21-23, Warsaw, Poland. Applied Science Reports 27, Polish Society of Animal Production 121-128.

Møller, S.H. 1999. Styring af minkproduktion (Management of mink production). Ph.D. Thesis, partly in Danish. Royal Veterinary and Agricultural University and Danish Institute of Agricultural Sciences. pp. 172.

Møller, S.H. & Sørensen M.T. 1999. Virkning af varierende foderstyrke på mængden af mælkekirtelvæv. (The effect of varying energy allowance on the amount of mammary gland tissue) I S.H. Møller (red) Temamøde vedr: Hvordan forbereder vi minktæver til parring, fødsel og diegivning. Intern rapport nr. 123. 49-53.

Møller, S.H. 2000. Langvarig restriktiv fodring af minktæver i vinterperioden er ikke en forudsætning for et godt hvalperesultat. Dansk Pelsdyravlerforening, Faglig Åsberetning, 1999, 59-65.

Møller S.H. & Chriél M. 2000. Health effects of feeding strategies in the pre mating and gestation periods of mink. Proceedings of the VIIth International Scientific Congress in Fur Animal Production. Scientifur 24, 4, VI 37-41.

Møller S.H. & Chriél M. 2001. Undgå fedtede hvalpe, undgå lav foderoptagelse sidst i drægtighedsperioden.(Avoid sticky kits – avoid low feed consumption during late gestation) Dansk Pelsdyravl 64, 193-196.

Møller, S.H., Hansen, S.W. & Sørensen, J.T. 2003. Assessing Animal Welfare in a Strictly Synchronous Production System: The Mink Case. Animal Welfare 12, 699-703.

Møller, S.H. & Sørensen, J.T. 2004. Mink production as a strictly synchronised animal production system: Identification of management problems and suggestions for relevant tools. Scientifur, 27, 3 (In Print)

NACMFC. 1998. National Advisory Committee on Microbiological criteria for Foods. Hazard analysis and critical control point principles and application guidelines. Journal of Food Protection. 61, 762-775.

Olesen, C.R. & Clausen, T.N. 1990. Fedtede hvalpe. Resultater fra Forsøgsfarmen Vest 1989. (Sticky kits. Results from research farm Vest in 1989). Dansk pelsdyravlerforening, Faglig Årsberetning 1989, 154-164.

Rattenborg, E., Dietz, H.H., Andersen, T.H. & Møller, S.H. 1999. Causes of mortality in mink collected from selected farms compared to mink submittet for laboratory diagnostic investigation. Acta. Vet. Scand. 40. 4. 307-314.

Schneider, R.R. & Hunter, D.B. 1993a. A survey of the causes of mortality in adult mink, with emphasis on the lactation period. Can Vet J, 34, 103-108.

Schneider, R.R. & Hunter, D.B. 1993b. Mortality in mink kits from birth to weaning. Can Vet J, 34, 159-163.

Sørensen, J.T. & Kristensen, E.S. 1992. Systemic modelling: A research methodology in livestock farming. In: (Gibon, A. & Matheron, G. et al. eds.): Global appraisal of livestock farming systems and study of their organizational levels: concepts, methodology and results. CEC proceeding eur 14479, 45-57.

Svennekjær, N.C., 1954. Minksygdomme og aktuelle problemer i minkavlen. (Mink diseases and current problems in mink farming). Dansk Pelsdyravl. 12, 384-404.

Tauson, A.H.; Elnif, J & Hansen, N.E. 1992. Energy metabolism and foetal growth in the pregnant mink (Mustela vison) Vth International Scientific Congress in Fur Animal Production. Norwegian Journal of Agricultural Science, Supp. No. 9, 261-267.

Tauson, A.H. 1993. Effect of body condition and dietary energy supply on reproductive processes in the female mink (Mustela vison). J. Reprod. Fert., Suppl. 47, 37-45.

Urlings, B. & Koenen, H. 2000. Disease and production management in mink farming. Proceedings from VIIth International Scientific Congress in Fur Animal Production, September 13-15, Kastoria, Greece. Scientifur 24, 4-VI, 27-31

II – 2 P

A preliminary linkage map of the mink (*Mustela vison*) genome

R. Anistoroaei[a], *K. Christensen*[a] *and A. Farid b*
[a] *The Royal Veterinary & Agricultural University, Division of Animal Genetics, Grønnegaardsvej 3, DK-1870 Frederiksberg C (Denmark)*
[b] *Department of Plant and Animal Sciences, Nova Scotia Agricultural College, Truro, Nova Scotia, B2N 5E3 (Canada)*

Abstract
The mink industry would benefit tremendously from linkage and cytogenetic maps of the mink genome, offering an opportunity to accelerate the rate of genetic improvement. The objective of this work was to create the first generation linkage map of the mink with at least 20 cM resolution, which will serve as a basis for further refinement. Genotypes of a mapping population consisting of four males, nine females and 71 F1 progeny were determined at 46 microsatellite loci, of which 34 were informative and could be scored accurately. Six markers were assigned to two linkage groups using the Crimap software. Physical mapping of the microsatellites was also performed using a panel of mink-hamster hybrid somatic cell lines, showing consistent results with the linkage map.

Introduction
Genome research is progressing at an astonishing pace and providing new frontiers in animal improvement. Genetic and physical maps for many livestock species have been constructed during the past decade (see for example http://www.thearkdb.org/). High density genetic maps, which are largely based on highly polymorphic microsatellite markers, have been used for the identification of genes that modulate monogenic traits, as well the identification of chromosomal regions which contain genes having a major effect on quantitative traits (QTL mapping). Despite the economic importance of mink production in northern Europe and North America, mink genomics research is lagging far behind other livestock species. A collaborative effort between the Royal Veterinary & Agricultural University in Denmark and the Nova Scotia Agricultural College in Canada is aimed at creating the first generation linkage and physical maps of the mink genome using available microsatellite markers. Here we present the preliminary results of this first attempt to create a linkage map for the mink.

Materials and methods
The mapping population: Seventy-one F_1 progeny from nine litters sired by four males constituted the mapping population. Two of the sire families were crosses between sapphire males and pearl females, and the other two were crosses between the Scand black and wild-type mink (mahogany). The main criterion for selecting a sire family was having at least 18 kits in three litters. Tissue samples (spleen and the lungs) were collected from sires, dams and kits after pelting.

Laboratory procedures: Genomic DNA was extracted from the spleens using a standard salting out method. Primer sequences for the amplification of 46 mink microsatellite markers by the polymerase chain reaction (PCR) were obtained from published sources (O'Connell *et al*., 1996; Brusgaard, 1998; Fleming *et al.,* 1999; Vardy, 2003; Vincent *et al.,* 2003, 2004). Several of these microsatellites were originally developed for other members of *Mustelidae* family and were shown to amplify mink DNA (Davis and Strobeck, 1998; Fleming *et al.,* 1999). Published microsatellite primers for otter (Dallas and Piertney, 1998) and badger (Carpenter *et al.,* 2003) were used, and those that amplified mink DNA and were polymorphic in our mink families were used in this study.
The forward primers were fluorescently labeled with NED (Applied Biosystems), 6-FAM or HEX (TAG, Copenhagen, Denmark). PCR amplification was performed in 10 µL total volumes containing 50 ng genomic DNA, 1 µM of each primer, 1-2.5 mM MgCl2, 0.2 mM each dNTP and 0,25 unit of *Taq* polymerase (Roche, Laval, QC). All loci were

amplified using the touch down PCR protocol (start 64˚C, 1˚ each step) followed by 33 cycles of 95˚C for 15 sec, annealing temperature (55-60˚C) for 60 sec and 70˚C for 15 sec in an Eppendorf Master Cycler (Hamburg, Germany).
Polymorphism of each locus was determined by genotyping the parents and a few offspring. The entire mapping population was genotyped using all the informative markers. Genotyping was performed using an ABI 3100 DNA sequencer equipped with the GeneScan and Genotyper software (Applied Biosystems, Inc., Foster City, CA). Diluted amplicons and a size marker (500 HD ROX, Applied Biosystems) were denatured at 90˚C for 3 minutes prior to loading (11 µL) onto 96 well plates. The Crimap software (Green, 1990) was used to perform two-point linkage analysis of the polymorphic loci.

Results

Of the 46 microsatellite loci that were tested, 34 (74%) were polymorphic in at least one sire family, eight were monomorphic and four did not produce stable allele sizes. A slightly larger number of loci were polymorphic in the mahogany families (33) than in the sapphire-pearl crosses (30). The two-point linkage analysis resulted in six LOD scores greater than 3.0 involving six loci (Table 1), which were classified into two linkage groups (Table 2). The results of the hybrid cell panel were consistent with the linkage data, but the characterization of the panel does not yet allow stating the chromosome numbers on which the linkage groups are located.

Discussion

The LOD scores indicated that the mapping population is useful in detecting linkages between loci that are up to 20 recombination units apart, and that a large proportion of the available mink microsatellite markers had high information content. Initial analysis suggests that our family material provides fairly high informativity on microsatellites among the 46 system used in the 3 panels, only 8 where non informative with no heterozygosity in the parent animals. The other 4 systems analyzed could not be read, however these may be optimized in the future. This report describes the preliminary results of a collaborative effort aiming at the creation of linkage and physical maps of the mink genome. One major limitation is the availability of microsatellite markers for mink. Approximately 200 more markers are needed to create a useful linkage map. While more microsatellites are being characterized at the Nova Scotia Agricultural College, we welcome researchers who wish to collaborate in this project, and we can share DNA samples of the mapping population.

Table 2. The two linkage groups with LOD scores greater than 3.0

Locus 1		Locus 2	RF*	LOD score
Lut604	-	Mvi248	0.09	3.41
Lut604	-	Mvi2243	0.04	8.13
Mvi248	-	Mvi2243	0.04	4.54
Mvi99	-	Mvi1404	0.18	4.46
Mvi99	-	Mvi1323	0.10	6.69
Mvi1404	-	Mvi1323	0.18	3.48

** Recombination frequency*

Table 1. Sequences of primers that were assigned to the two linkage

Loci	Reference	Accession number	Primer1	Primer2
Mvi1323	Vincent et al., 2003	AF480849	AATGGGGGAATTTACAGGT	CTGAAATACAAGGGCATTCTT
Mvi2243	Vincent et al. 2003	AY053518	CGGACATTTGTTCTAAGAGGT	AGATTAACAAGCCATGCTC
Lut604	Dallas & Piertney, 1998	Y16300	GAGATGGAGCCATATGTTGGA	TTTTCAACAATTCATGCTGGA
Mvi1404	Vincent et al, 2004	AY249175	CCTGCTTTTCTCCTATCCATT	GGGGTAGAACACAACGTTTTC
Mvi248	Brusgaard, 1998	U87255	CCGGGGATCTTTTTCTCTTC	TCAGCAAAGTGTGGGATGAA
Mvi099	Fleming et al. 1999	AF132106	AGAAGAGAGCAGAGGCATCA	GATGAGGAGGGATGTTGAGC

References

Brusgaard K. Establishment of a preliminary genomic map of the American mink *(Mustela vison*). 1998. Dissertation in Danish. Danish Agricultural Research Center, Foulum, Denmark.

Carpenter P.J., Dawson D.A., Greig C., Parham A., Cheeseman C.L., Burke T. 2003. Isolation of 39 polymorphic microsatellite loci and the development of a fluorescently labeled marker set for the Eurasian badger (Meles melses) (Carnivora: Mustelidae). Mol. Ecol. Notes 3: 610-615.

Dallas, J.F. and Piertney, S.B. 1998. Microsatellite primers for the Eurasian otter. Mol. Ecol. 7: 1248-1251.

Davis, C.S. and Strobeck, C. 1998. Isolation, variability, and cross-species amplification of polymorphic microsatellite loci in the family Mustelidae. Mol. Ecol. 7:1776-1778.

Fleming, M.A., Ostrander, E.A. and Cook, J.A. 1999. Microsatellite markers forAmerican mink *(Mustela vison*) and eramine (*Mustela erminea*). Mol. Ecol. 8: 1351-1362.

Green P, Falls K., Crooks S., 1990. Documentation for CRI-MAP version 2.4.

O'Connell, M., Wright, J.M. and Farid, A. 1996. Development of PCR primers for nine polymorphic American mink *Mustela vison* microsatellite loci. Mol. Ecol. 5: 311-312.

Vardy, T.L. 2003. Structure and sequence variation of the mink prolactin gene. M.Sc. thesis, Nova Scotia Agricultural College, Truro, NS, Canasa, p102.

Vincent, I.R., Farid, A. and Otieno, C.J. 2003. Variability of thirteen microsatellite markers in American mink *(Mustela vison*). Can. J. Anim. Sci. Vol. 83: 597-599.

Vincent, I.R., Farid, A and Sun, G. 2004. Isolation of microsatellite markers from AG, AC and AGC enriched libraries of the American mink (Mustela vison) genome. Submitted.

II – 3 P

Not diagnosed stage of Aleutian Disease

V.S. Slugin

Abstract

The purpose of researches - to establish the cause of mass cases of negative reaction of a counter immunoelectrophoresis (CIEP) and iodine-agglutination test (IAT) at the kits from positive reacting the mothers, i.e. to determine the cause of abaissement (disappearance) of positive reaction at infected by a aleutian disease (AD) virus (ADV) of mink. Simultaneously investigated by methods CIEP and IAT more than 100 thousand blood samples at adult mink various colours and at their pups in conditions of the largest farm of Russia with AD. Besides we exposured with ADV by different methods of the adults females and their offsprings (about 300 animals) and with the help CIEP-test studied dynamics of accumulation antibodies against ADV and its titers on an extent from pregnancy up to 10-months age and sometimes more. In necessary cases we made laparotomy for study of an opportunity of transplacental transfer of a ADV. Also we observed efficacy of exposure and course of AD.

The mass cases (almost up to 70 %) of a petering antibody against ADV at the kits, infected intrauterine or in the first birthdays from mother (or stepmother) are fixed. The abaissement descends soon after weaning and often remains until autumn. At about 15-20% of animals antibodies to ADV to be absent in the course several months.

The stage of development ADV is fixed temporarily inaccessible to diagnostics by means CIEP and IAT. The cause it is particulate mainly temporary tolerance of the pups and in the certain measure colostric antibodies in case of vertical infection.

Introduction

It is well-known, that AD is present on many farms of the world. Its erradication sometimes becomes the large problem because of impossibility of early revealing all infected mink. One of the causes of unsuccessfulness of diagnostics by means of CIEP are the mass cases of absence of positive reaction at the kits, birth by the infected mothers. So, by us was fixed, that multithousand stock of the females with positive CIEP brings offsprings, at which the reaction becomes negative to the moment of autumn researches of blood samples (table 1).

The date of table 1 testify to natural abaissement (petering) of positive reaction at an appreciable part of animals - at 1,2-18,4%. The most infrequent abaissements (1,2 %) were at mink with signs of disease (empty, abort and with dead kits), whereas at healthy - often (8,9-18,4 %). But unexpectedly has appeared, that at scheduled inspection mink (tab. 2) the incidence among the kits was much below (in 1,4 - 112,5 times), than at the adult.

This difference, probably, also would not require any explanation, if the conditions of the contents, feeding, service both adult and joung growth mink by something differed. But they had a uniform ration and the same food, kept in the same sheds and served the same workers. Means, the differences in prevalence of the kits and adults were caused not by an external environment, and any other factors influencing result of researches.

Practically at all - it is possible to present, that in conditions when almost 50% of parent herd of genotypes AA* and kkpp was infected with ADV, the kits could not exposure from the mothers, being not in isolation from the mothers.

Tab. 1. Reproducibility of positive CIEP at repeated blood analysises spontaneously infecting AD mink

Interval of researches (month)	Is taken mink	The positive CIEP has repeated	
		Amount of cases	%
0,5	303	276	91,1
4-5	86	85	98,8*
9	505	412	81,6
Total	894	773	86,5

**-mink with signs AD.*

Tab. 2. Illogical interrelations of a level positively reacting on AD of the kits mink and their parents at simultaneous research.

Is investigated mink		Positive results, %					
		IAT		The incidence of the adult to the kits	CIEP		The incidence of the adult to the kits
		Adult	Kits		Adult	Kits	
Total							
Different types	38257	1,8	1,3	***1,4***			
	35041	13,4	7,0	***1,9***	26,9	12,7	***2,1***
Including some genotypes							
kkpp, mmaa	3856	0,5	0,04	***12,5***			
aapp	2813	6,0	0,3	***20,0***			
aapp	3418	4,5	0,04	***112,5***			
ÀÀ*	1365				42,6	14,9	***2,9***
kkpp	4072	42,7	1,1	***38,8***	52,4	2,3	***22,8***
AA	4522	5,0	4,8	***1,04***	27,7	8,3	***3,3***

kkpp - pearl american 2-recessive, mmaa - lavender, aapp - sapphire, AA - standard dark brown, AA - standard black.*

Thus, the date of tables 1 and 2 yield the establishment to search for the cause of abaissement of a positive take of reactions and illogical interrelations of quantity of reacting kits and their parents first of all in the immunological answer of an mink organism. For finding - out of this question the present researches also were undertaken.

Material and Methods

Simultaneously investigated by methods CIEP and IAT more than 100 thousand blood samples at adult mink of various types (colours) and at their kits in conditions with AD of the largest farm of Russia (the brightest materials are submitted in tables 1-2). CIEP and IAT put on our method (1982). Besides infected with different methods of the adults mink and their offsprings (about 300 experimental animals) and by CIEP-test studied dynamics of accumulation antibodies to a ADV on an extent with intrauterine exposure about 10 months and sometimes more. In necessary cases made a laparotomy for study of an opportunity of transplacental transfer of a ADV and antibodies to fetus. Observed efficacy of exposure and courseAD.

Results

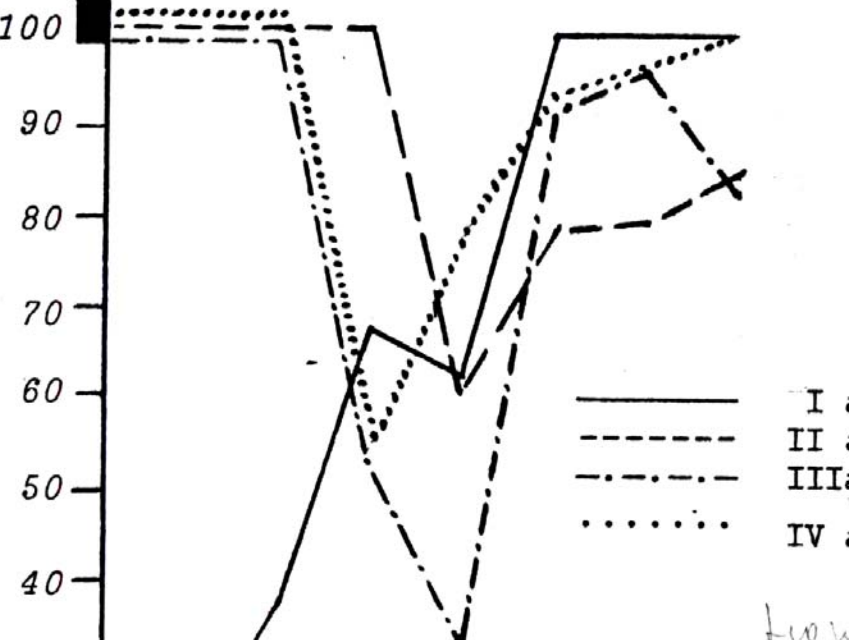
In tables 1-2 the numerous cases of discrepancy of positive reactions (IAT and CIEP) at the adult mink and their offspring are shown. The study of dynamics of accumulation specific antibody has allowed to find, that after weaning at the majority of the kits from under infected of the mothers in the certain season petered positive CIEP (fig. 1).

The pups I-II and III-IV of groups descended accordingly from the same letters.

The specific antibodies were found in all experimental kits at once after birth or later (fig. 1), that specifies a high contageousness AD in this season. In passing it is necessary to notice, that in the given experiences 83,3 % females has exposured after indroduction into the nest of infected kits; characteristicly, that for 30,2 % of the kits and 33,3 % infecting females was deadly within 6 months with AD.

In a fig. 1 it is visible, that after weaning season (40-60 days) at the kits II-IV of groups antibodies was detected very rarely (30, 50 and 60 %), though up to the specified term all 100 % of the kits of the named groups were seropositive. The abaissements positive CIEP descended not unsystematically, and to the certain law. So, at contact infection in I group, where there was no influence colostric specific antibody, positive CIEP has appeared since the 20-th day and practically did not drop out; to 120-th day and later all 100 % of the kits become seropositive. At the kits from the same litters with them (also seronegative at birth), introducted into nests to seropositive females at once after birth (II groups), CIEP has become positive since the first day, but since the 60-th day of its abaissement were often (60 %). Besides in this group the petering of antibodies on the end of researches in high percent

Fig. 1. Dynamics of revealing specific antibodies at mink after intraplacental exposure or in early posthanal season.



a) 0-180 - age of the kits (days), b) 0-100 - percent of the seropositive pups at date of inspection, c) ***I*** *group - contact exposure (infection) of the healthy kits from the seropositive kits, enclosed into a litter,* ***II*** *- the healthy kits are enclosed to the reception seropositive mother (stepmother) and into her litter,* ***III*** *- transplacental infection from the experimentally infected mother (contents of the kits under the seropositive mother),* ***IV*** *- transplacental infection, but contents of thekits under the reception seronegative mother (stepmother).*

(16 %) is marked also. Pays attention, that at the kits III group, infecting intrauterine and receiving the milk of the seropositive mother, the abaissements CIEP were most appreciable (67 %) and long (4 months), whereas at the kits from the same litters with them, but under the seronegative reception mother, though there were often abaissements for the 60-th day, but to the end of experience CIEP was as in I group (100%).

Abaissements positive CIEP on 60- and the 90-th days of life of the kits, as it is visible from a fig. 1, are connected appreciably to a transplacental infection or with consumption of milk seropositive female, i.e. with colostric specific antibodies. The combination of consumption colostric antibodies with intraplacental exposure enlarges percent and duration of abaissements. The titration antibodies at mink of these groups specifies also fall of a level antibody after weaning season. Thus the fall of titers antibody has not touched the kith I group.

Discussion

For the first time is proved, that the vertical exposure (intrauterine and per the first day of life through milk feeding female) is accompanied by mass cases (up to 70 %) petering positive CIEP at the kits from the moment of them weaning from the mother (45-day's age) and up to 120-day's age proceeds. Then majority of the infecting kits become seropositive, but part from them (15-20 %) to autumn check of a blood does not yield positive reaction, though, as have shown other our experiences, contain a pathogenic virus. At the kits, infecting at contact, but bringing up seronegative

females, the positive reaction is saved practically at any time of year, and antibody level highest, that corresponds to progressing form AD. Thus, two factors influencing petering positive CIEP are revealed is a transplacental infection and colostric antibody. As the combination of these two factors yields highest percent of abaissements and greatest duration of abaissements, there is an establishment to speak about primary influence of tolerance (unresponsiveness) and additional influence colostric antibody. However in this case tolerance does not protect of mink from disease and death, hence, she is not complete (only particulate). Colostric antibodies influence by a similar mode, but their influence is less expressed and shows much later.

The law, revealed by us, has the immediate attitude to practice, is especial in unsuccessful farms, where the breeding herd has exposured befor labour. First, it is impossible to investigate the kits before 120-day's age. Secondly, at autumn check (October - November) about 15-20 % infected of the kits fails to be found by a method CIEP, therefore it is necessary first of all to investigate the adults mink. In case of positive reaction at females it is necessary to remove from herd their kits without carrying out of researches (certainly, delete and females). Thirdly, the opportunity of improvement of a farm becomes inconvenient. In such situation the best yield is research of all families mink (female plus her kits), from which it is planned to leave on breeding aims even one animal.

The carried out researches have revealed two causes causing the inapparent or the progressing forms AD, is a vertical and horizontal exposure.

Conclusions

1. At vertical exposure temporary particulate tolerance and in the certain measure colostric antibodies the mass cases of petering positive CIEP at infected of the kits mink cause, that allows to speak about presence of a not diagnosed stage of development of an AD.
2. The first proof of holding vertical infection on a farm is the excess of a level prevalence of the adult mink above the kits.
3. The horizontal infection is accompanied by progressing development AD, accessible diagnostics at any time of year, thus the level of the positive reactors kits is higher, than adult.
4. The not diagnosed stage AD demands updating erradication measures in infected farms.
5. Colostric antibodies do not protect the mink from exposure and death.

References

Slugin V.S. – Aleutian Disease of mink and route of its erradication. - Dissertation of the doctor of veterinary sciences. - Moscow, 1982.

II – 5 P

Characteristics of some morphological and biochemical indices of marmots bred in cages

Galina A.Fedoseeva, Elena A.Tinaeva*, Nikolai A.Balakirev*, Igor A.Plotnikov**, Nadezhda A.Suntsova***, Nikolai N.Shevlyuk*****

** V.Afanasiev Research Institute of Fur Bearing Animals and Rabbits*

Ramensky District, Moscow Region, Rodniki 140143, Russia;

*** Russian Research Institute of game Management and Fur Farming, Kirov, Russia;*

**** Academy of Agriculture, Vyatka, Russia;*

***** Academy of Medicine, Orenburg, Russia*

E-mail: NIIPZK@orc.ru

Abstract

This research is the main part of series of experiments, directed to the creation of a population of species of Marmots (Marmota bobac) bred in cages on the state pedigree farm Pushkinskiy, situated in Moscow region.

Its aim was the evaluation of haematological indices: haemoglobin, erythrocytes and leukocytes, indices of blood coagulation and selected chemical indices: total protein and its fractions, lactatdehidrohenasa, triglycerides, ceruloplasmin in marmots bred in cages.

With the use of macroscopic, histological, histochemical and electro-microscopic methods characteristics of organs of reproductive systems were studied. It was found out that males of marmots before hibernation have a serious reconstruction of testicles, which is followed by the enlargement of their weight (almost in 3 times). There was also registered activation of endocrine and herminative structures of testicles before the beginning of hibernation. In female marmots there were found processes of activation of herminative function of ovaries.

Introduction

The Development of cultivation of genus marmots (Marmota) and the development of technology of the cage maintenance of these animals are proved by the necessity of expansion of perspective objects of cage fur farming and preservation of disappearing kinds (Mashkin V.I., 1997).

The perspectivity of introduction of marmots into zooculture is caused by their fast adaptation to a person, herbivorousness and, certainly, semi-annual hibernation.

The Studying of marmots' reproductive activity is important not only in the theoretical plan, but also due to the connection with economic use of these animals because the exposure of biological laws of dynamics of reproductive activity, spermatogenesis and estral cycles can increase the results of reproductive ability.

A number of features of these animals (short period of reproductive activity, large range of morphofunctional variability of reproductive system, large sizes) makes them very convenient biological object for the research of morphofunctional characteristics of reproduction.

Till now the questions of histophisiology of genital glands of males and females of marmots by the end of hibernation of these rodents and during the preparation of animals for pairing are almost not studied. There are no morphological, physiological and biochemical data on the condition of genital glands of marmots not only by the end of hibernation, but also during it. The given period in the condition of genital glands remains unexplored. Therefore we decided to carry out such work.

Material and Methods

For supervision over the development of estral cycle (stages of desire and ovulation), alongside with the estimation of external change of a loop and animals' behavior a method of vaginal smears. Smears were taken from the lateral arch of the top third of vagina with the help of a wadded tampon on a match. After fixing by methyl spirit, smear was painted with Lefler blue and paint of Romanovskii-Gimza which has improved its visibility under a microscope. According to the change of a smear objective criteria of an estimation of cellular elements and a parity between them (an index of maturing) were determined. The index of maturing expresses the parity between basal, superficial and intermediate cells.

For taking blood original technique was used. A marmot was fixed in the machine by neck with the

use of a metal arch. A tourniquet was imposed in the area of a hip Blood was taken by a spear-shaped needle from medial vena in the proximal parts of a shin. To avoid haemolysis, blood flowing from a needle was collected in a test tube without use of a syringe.

By this technique blood was taken from marmots, both in active condition, and during the period of hibernation when body temperature was +7°C.

The level of hemoglobin, leukocytes and erythrocytes was defined with the use of standard methods. General fiber - biuret method.

Digital materials of experiments were processed by a method of variational statistics (Plohinskiy, 1980) with use of applied computer programs.

Studying morphofunctional features of reproductive system of females and males of marmots was carried out on histologic preparations received from animals during slaughter, prepared with the use of standard methods.

Results and discussion

Results of measurement and weightings of internal tissues, received during the slaughter of animals, are submitted in tables 1,2 and 3.

In the period of hibernation blood was taken for researches from two females and four males in the age of 6 months. The data are submitted in table 5.

Before the hibernation of animals there is an essential structural and functional reorganization of testicles, accompanying significant (almost 3 times) increase of their weight. In gonads the square of intersticial tissues decreases due to the increase of the area of tubuli seminiferi convoluti. In spermatohene epithelium of tubuli seminiferi conv processes spermatohenesis become more active. In this period endocrine structures become more active. The sizes of endocrinocites and their nucleus are sharply increased. The volume of nucleus of Leydig cells has made 98,3 ± 3,7 microns. In a population of endocrinocites there grows the number of mature functionally active cells. On the level of light optics these cells show significant development of cytoplasm, large nucleus with large maintenance of euchromatic structures. Their ultrastructure is characterized by good development of smooth endoplasmatic reticulum, occupying the most part of cytoplasm, a lot of mitochondrias and lipid inclusions of cytoplasm.

There was carried out the research of some parameters of blood coagulation system, and of lipid peroxidal oxidation of males and females of marmots during the hibernation in the age of 6 to 18 months. The amount of protrombin has made 100 КВИК %, or 0,89 INR, activational partial trombine time - 67,6 sec and fibrinohene - 3,8 g/l. Ceruloplasmin contains in the amount of % of 39,9 mg, tiogroups (SH groups) - 3,6 **ммоль**/l, malone aldehyde (MDA) - 4,4 **мкмоль**/l.

Conclusion

The received results allow to speak about the remarkable feature of the reproduction of marmots - essential activisation_of endocrine and herminative structures of testicles before the hibernation. One of the reasons of spermatohenesis activisation in tubuli seminiferi convoluti can be the previous increase of secretion activity of testicular endocrinocites.

The biological meaning of this phenomenon is the preparation of reproductive organs for processes of reproduction which begin in marmots after the exit of animals from a hibernation.

During preparation for the beginning of hibernation (July - August) in females of marmots there reveals processes of activization of herminative functions of ovaries. In ovaries processes of activization of growth of follicles were marked. Morphological equivalent of this process was the increase of average sizes of growing follicles (in comparison with the sizes of similar structures in ovaries in May - June), the strengthening of proliferational activity of follicular epithelium and the increase of sizes of follicular cells. Probably, these processes are directed on the preparation of females’ genital glands to the duplication in the following season. This phenomenon (activization of herminative functions of ovaries) can be connected to the compressed period during which ovulation and pairing take place.

References:

Mashkin V.I. 1997. European marmots: ecology, preservation and use. Kirov, 156 p.

Plohinskiy N.A. 1980. Algorithms of biometrics. P. 67-74.

Table 1. Weight of organs of marmots, g

Sex	n	Kidney		Heart	Liver	Spleen	Gall bladder
		left	right				
Females	9	12,87±0,95	14,03± 0,93	15,9±0,61	164,6±12,3	9,43±1,29	8,80±1,29
Males	8	18,3±1,51	18,1±1,80	19,1±1,97	184,1±13,7	8,32±1,45	7,74±1,24

Table 2. Morphological rates of females reproductive organs of 18 months age marmots

	Of ovaries				Oviduct				Body of uterus	
	n	left	n	right	n	left	n	right	n	
Length, mm	8	46,0±10,6	6	43,5±10,1	8	185,0±9,0	8	183,3±12,6	9	358,1±3,92
Weight, mg	8	41,3± 8,95	6	31,3±4,81	8	962,5± 316,4	8	1065,0±143,7	9	2230,2±281,2

Table 3. Morphological rates of android glands of 18 months age marmots

	n	left	n	right
Length, mm	8	17,9± 1,63	8	17,9± 1,51
Weight, mg		2012,5± 254,4	8	2033,8± 265,3

Table 4 Biochemical rates of serum of 18 months age marmots.

Rates	n	M±n	δ	Cv	lim
Total protein, g/l	7	77,94± 3,29	8,71	11,2	63,75-86,25
Triglycerides, mg/100ml	6	58,44± 7,53	18,45	31,6	43,54-88,21
Laktatdehydrogenase, (ЬДН), ие/л	5	269,99± 37,41	83,66	30,9	150,00-349,99
Albumins, g %	7	31,19± 2,55	6,76	21,7	24,24-44,82
α- Globulin, g %	7	26,31± 2,97	7,85	29,8	18,00-36,36
β- Globulin, g %	7	22,98± 1,61	4,26	18,5	17,24-30,00
γ- Globulin, g %	7	19,52± 2,09	5,55	28,4	11,91-26,92

Table 5. Blood rates of 6 months age marmots (hibernation)

Rates	Females	Males
Hemoglobin, g/%	17,3±0,65	16,1+ 0,99
Erythrocytes, mln./mm^3	4,34 ± 2,00	5,58 ± 2,39
Leucocytes, th./mm^3	4,62 ± 0,75	6,30±0,92

II – 6 P

A level of some indices of the oxidation state in blood plasma of mink at slaughter period under the definite maintenance and feeding conditions

Hanna Bis-Wencel , Leon Saba, Adam Liczmański[1], Bożena Nowakowicz-Dębek
Laboratory of Reproduction Biology of The Department of Animal and Environment Hygiene, The Faculty of Biology and Animal Breeding, UA in Lublin, ul.Akademicka 13,20-9510 Lublin,
E-mail: hanka13@poczta.onet.pl , POLAND.
[1]The Department of Toxicology, The Faculty of Biology and Animal Breeding, UA Lublin, 20-950 Lublin, ul.Akademicka 13, POLAND.
**This work was conducted as part of the research project No. PO6Z 0512 25 financed by the State Committe for Scientific Research (2003-05)*

Summary
The oxygen radical excess is hazardous for animal health due to their high nonspecific reactivity. It may the imbalance between antioxidants and prooxidants in favour of the oxidation, that results in so called oxidation state occurrence. The objective of the present work was to determine values of some blood plasma parameters considered the oxidation state markers in the minks aged one year and maintained at the definite farm conditions. The investigations were performed at the mink farm "C" situated in the south eastern part of Poland. The yearlings chosen for the experiment were meant for slaughter. Blood was collected twice in December. It was taken from heart of 60 minks. The examinations on blood plasma covered determination of soluble protein level, glutathione peroxidase (GPx), glutathione reductase, superoxide dismutase (EC-SOD) and total glutathione strength (GSH-GSSG). Moreover, there was established total oxidation activity (TAS).

Introduction
Excess of aerobic radicals is hazardous for animal health owing to their high nonspecific reactivity. This may lead to the imbalance between antioxidants and prooxidants in favour of oxidation, that in a consequence induces the status called the oxidation stress. Numerous clinical examinations confirm a relation between the oxidation stress and health state disturbances [Kleczkowski et al.,1998; Saba et al.,1993; Saba et al.,1996]. The mentioned above state need not to be caused by a direct influence of free radicals, still it is of great importance in the whole chain of events giving rise to a polyetiological disease and development of the concurrent symptoms. Thus, the sources of free radicals seem to be vital as they are developed in organism under the endo- and exogenous conditions. The latter include, among others nutrition, ultraviolet radiation and substances polluting the environment predominantly [Bartosz,2003; Benzie &Strain.,1996; Sitarska et al.,1997].
The objective of the present paper was to determine the values of some blood plasma markers considered the oxidation state indices at minks aged 1 year maintained at the definite farm conditions.

Material and Methods
The investigations were carried out at the "C" farm situated in the south-eastern part of Poland. The object was surrounded with a broad green belt constituting a natural barrier for odours not to spread away. Stock of the basic pack was around 500 females. The yearlings chosen for the examination were meant for slaughter. Blood was collected from the heart of a mink of a fine variety "scan brown". In plasma there were determined: total glutatione concentration (GSH-GSSG) according to Akerboom and Sies` method [Bartosz,2003], activity of superoxide dismutase (EC-SOD) after the adrenaline method of Misra [Bartosz,2003], glutatione peroxidase activity (GPx) and total antioxidant status (TAS) with a diagnostic test of RANDOX, a level of soluble protein with Lowry`s method [Bartosz,2003].The mentioned above determinations were performed by the two-beam spectrophotometer CE 7200 CECIL.
The obtained results were analysed statistically using the ANCOVA method.

Results
Glutatione (GSH+ GSSG) belongs to the antioxidants operating on the basis of the non-enzymatic mechanism. A glutatione thiole group readily reacts with free radicals, the fastest with hydroxyl radical and a bit slower with organic

radicals occurring at the aqueous phase. The reactions of glutatione and free radicals of organic substances, in particular proteins, may result in the "repair" of these particles in favour of formation of glutatione free radical [Bartosz,2003]. A level of mean values (GSH+GSSG) at the examined minks developed within 0.103 – 0.218 U/l interval.

A vital role in the defensive system against the FR attack was performed by the antioxidative enzymes. They include, among others superoxide dismutase (EC-SOD) and glutatione peroxidase (GPx).The above mentioned enzymes metabolite free radicals ($O^{\cdot}_2$ in the case of SOD) or the half products (H_2O_2) in the case of (GPx) into less toxic or nontoxic products. Activity of superoxide dismutase in the extracellular fluids is lower than in intracellular. Despite this, the cell surface is protected against superoxide anionoradical by means of small quantity of EC-SOD bounded with them [Bartosz,2003]. Throughout the investigations, the EC-SOD level reached 20.05 – 10.23 U/l.

Glutatione peroxidase (GPx) is an adaptative enzyme, its activity increases in response to the oxidation stress [Sies,1985].It catalyzes the hydrogen peroxide reduction and organic peroxides by the reduced glutatione. The mean levels of glutatione peroxidase in minks ranged from 21605.31 – 1879.60 U/l.

A response to the oxidation stress manifests itself with the definite antioxidation state that can be presented as the total activity (TAS). It is likely to be a perfect marker of this state as there are numerous interactions recorded between the antioxidants that are easily overlooked being individually determined. What is more, it is impossible to determine all the antioxidants because a part of them has not been identified so far. The total oxidative activity of minks oscillated at the level of 0.605 – 0.575 U/l. The mean values of soluble protein ranged 79.38 – 85.08 mg/ml.

Tab.1. The mean values of the blood oxidation stress parameters of mink.

Parameters	Collection I	Collection II
Glutatione peroxidase(GPx) U/l	21605.310	18796.600
Glutatione Reductase U/l	129.270	80.970 **
SuperoxideDismutase EC-SOD U/l	20.050	10.230**
Total Glutatione GSH-GSSG U/l	0.103	0.218 *
Total Antioxidant Status (TAS) U/l	0.605	0.575
Protein g/l	79.380	85.08

** p≤0,05, ** p≤0,01*

Discussion

Glutatione participates in the reconstitution of the damaged cell components. It is noteworthy that its main function is to keep protein thiole groups reduced, which in many cases is simply essential for the functional activity of proteins. What seems interesting is a fact that its concentration drop to only half of the values regarded the references, as a rule does not lead to any noticeable physiological effects. Whatsmore, the enzymes interacting with GSH in a cell do not change their activity even under the conditions of a significant decrease of this tripeptide. It is only a considerable fall of GSH concentration that reduces their efficiency [Bartosz,2003]. In the carnivorous furry animals breeding, in that minks, there appears a special risk of animal organism exposure to the oxidation stress resulting from air pollution. It follows from a fact that furry animal farms emit some tens of odourforming substances, mainly sulphoorganic compounds, ketones, aldehydes, alcohols as well as aliphatic and aromatic hydrocarbons [Nowakowicz-Dębek et al., 2001; Saba et al.,1993; Saba et al.,1996].

These compounds are characterized not only with noxious smell but quite frequently these are toxic or carcinogenic substances for man and animal. It often happens that EC-SOD is termed a " locally specific" protective enzyme. It is connected with the analogy to a local specific development of radical OH [Bartosz,2003; Makurland ,1986].

In human cells there was detected appearance of four different forms of glutatione peroxidase. The first one is intracellular, called classical (CGPx). It occurs in different cells, among others in erythrocytes and its function is the protection of cells against the oxidation stress, especially hydrogen peroxide. The next form is GI-GPx, i.e. gastrointestinal peroxidase recorded in the alimentary tract walls.[Brigelrius-Flohe et al.,2001]. Besides, its presence is also detected in the liver and some lines of neoplastic cells. It makes the barrier against the peroxides and xenobiotics

that entered the alimentary tract. The volatile gaseous substances are generated at a farm not only during the complex digestive processes recording in the stomach. They are also made due to intricate decomposition processes in discharges falling down or surged under the cages. Moreover what seems important is the impact of high-energy and high-protein feed like, gurry or meat scraps [Nowakowicz-Dębek et al.,2000;2001.:]. A plasma form (pGPx) occurs mainly in the extracellular fluids and in tissues, which are in contact with them (kidneys, placenta). The last form is glutatione peroxidase of phospholipide hydroxides (PHGPx).The greatest amount of this enzyme in mammals is detected in male testes.It is interesting that occurring in the sperm cell mitochondria, it is responsible for nearly half of total protein content of external mitochondrial membrane of sperm, while it is inactive in mature sperm cells. No doubts, it has a significant physiological function there as in the sperm cells of infertile man substantially smaller quantity of this enzyme is detected [Bartosz,2003].
Owing to a lack of publications with the reference values, the values enclosed may be treated as preliminary studies.

References

Bartosz.G.,2003.:The other face of oxygen; PWN; Warszawa .

Benzie I.F.F., Strain J.J.,1996.: The ferric reducing ability of plasma (FRAP) as a measureof „antioxidant power”: the FRAP assay, Anal. Biochem. 239, 70-76.

Brigelrius-Flohe R., Muller C., Menard J., Florian S., Schmehl K., Wingler K.,2001.: „Functions of GI-GPx: lessons from selenium – dependent expression and intracellular localization”; Biofactors 14; 101-106.

Kleczkowski M., Kluciński W., Sitarska E., Sikora J.,1998.: The oxidation stress and some undices of the animal oxidation state; Medycyna Wet. 54; 166-171.

Nowakowicz-Dębek B., Saba L., Bis-Wencel H.,2000.: Emissionen aus Pelztierfarmen in Polen; Agrarforschung, 7 (2).

Nowakowicz-Dębek B., Saba L., Bis-Wencel H.,2001.: Zanieczyszczenie powietrza alkoholami, aldehydami i ketonami przez fermy zwierząt futerkowych.; Medycyna Wet. 57 (5); 346-348.

Makurland S.L.,1986.: Ceruloplamin, extracellular-superoxide dismutase, and scavenging of superoxide anion radicals.; J. Free Rad. Biol. Med.2; 255-260.

Saba L., Bis-Wencel H., Sławoń J., Polonis A.,1993.:Air and soil pollution by carnivorous furry animal farms. Ann.;UMCS Sect., vol.11;215-222.

Saba L., Sławoń J., Tymczyna L., Bis-Wencel H.,1996.: Sulphoorganic odour emission from carnivorous furry animal farms. Ann.; UMCS Sect.,; vol.14; 177.

Sies H.,1985.: Oxidative Stress: introductory remarks. Sies H. (ed): Oxidative stress, Academic Press; 1-8; London.

II – 7 RP

Mink Nursing Sickness Survey in North America

K. Rouvinen-Watt and A. M. Hynes
Canadian Center for Fur Animal Research, Nova Scotia Agricultural College, Department of Plant and Animal Sciences, Truro, Nova Scotia, Canada Email: krouvinen@nsac.ns.ca

Abstract
Nursing sickness is a metabolic disorder of large economic importance to the mink industry. Fifty-two (52) North American mink ranchers, housing a total of 99,333 breeder females with a production of 393,717 pelts, responded to a nursing sickness survey. Forty-five (45) % reported problems with nursing sickness with 5-40% of females affected. The farms with nursing sickness had a higher litter size and housed their females more often in multi-row sheds with more southern exposure. Water cups were employed more often as the sole source of water on farms with no problems with nursing sickness. On healthy farms, breeder female selection focused on body length, whereas weight was emphasized on farms with nursing sickness. In addition, on healthy farms, breeder female conditioning begun during the fall, and included keeping the females active. Weaning occurred more often around seven weeks on farms with problems, whereas the healthy farms weaned either earlier or later. The farms without nursing sickness fed much more fish in their diet (34-42%) throughout the production year than the farms, which encountered problems (18-27%). Several on-farm management practices were identified, which promoted better glycemic control in the lactating females. These practices appear to be strongly associated with the reduced occurrence of nursing sickness.

Introduction
Nursing sickness is a metabolic disorder of large economic importance to the mink industry and represents the largest single cause of mortality in the adult female mink (*Mustela vison*). It is believed to develop from a complex of metabolic, nutritional and environmental factors, which influence the ability of the mink dam to meet the extreme demands of lactation (Clausen *et al*. 1992). Increasing dam age, large litter size, and female weight loss have been identified as major determinants for the development of nursing sickness (Clausen *et al*. 1992). An increase in demand for gluconeogenesis due to heavy milk production may also be a predisposing factor as abnormally high levels of plasma glucose have been observed in the affected dams (Wamberg *et al*., 1992). While the etiology of nursing sickness remains uncertain, it has been suggested that it is linked to a disruption in glucose homeostasis (Børsting and Gade, 2000). It has been proposed by Rouvinen-Watt (2003) that the underlying cause of nursing sickness is the development of acquired insulin resistance with obesity (or lipodystrophy), n-3 fatty acid deficiency, and high protein oxidation rate identified as key contributing factors. Genetic susceptibility, diet, energy and fluid deficit, and stress are associated factors, which may further contribute to the development of the disorder (Rouvinen-Watt, 2003). With morbidity as high as 14-15% and mortality up to 8% (Clausen *et al*. 1992), differences in incidence rates observed among individual ranches demonstrate the importance of ranch-level factors in the development of the disease (Schneider and Hunter, 1992). The objective of this study was to improve our understanding of the on-farm factors, by conducting a survey among mink producers in North America on their ranch history and animal and feeding management practices, that may contribute to the occurrence of nursing sickness.

Materials and Methods
A survey was sent out to mink ranchers in Canada and the US during the spring of 2002. The census consisted of all licensed mink ranches located throughout North America and the surveys were administered by mail. Survey completion was on a voluntary basis and prepaid envelopes for return mailing were enclosed. The study used a three-part, ten-page survey, designed to collect background data on individual ranch history, animal management and feeding practices with easy-to-interpret short-answer questions or categorical responses requested. The

ranch history section of the survey examined, for example, the occurrence and frequency of nursing sickness on the ranch, the type of animal housing, orientation of buildings, roofing material, pen and nest box sizes and watering systems. The animal management practices surveyed consisted of breeder selection criteria, animal conditioning for breeding, handling of females and weaning methods. The feeding practices section focused on diet composition during the different stages of the production cycle, as well as feed location and feeding frequency during the nursing period. Based on reported problems with nursing sickness, the survey population was divided into two groups: nursing sickness (NS) ranches and healthy (H) ranches, which were statistically compared regarding the surveyed parameters. For categorical data, the frequency distributions (e.g. breeder selection criteria, weaning method) were tested using Fisher’s exact test, whereas continuous variables (e.g. litter size) were analyzed using Proc GLM in SAS (1999) and regression analysis was done in SigmaPlot.

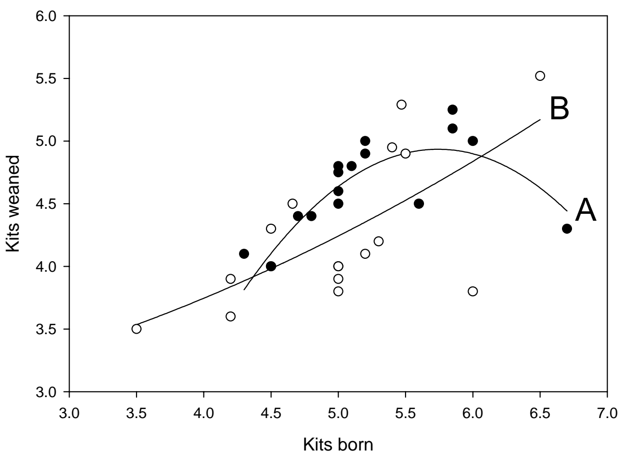
Results

Overall, 52 ranches responded, consisting of 27 from Canada and 25 from the US. Of the total respondents, 13 surveys were found to be ineligible due to census errors (8 cases), non-response (4 cases) and changes in farm operation status (1 case). Census error refers to those surveys not clearly indicating ranch nursing sickness status. These respondents were excluded from the comparative analyses, as they could not be assigned to either the nursing sickness (NS) or healthy (H) category.

Ranch History

The survey respondents housed a total of 99,333 breeders females of which 56% represented the black, 31% the brown, 8% the blue and 5% other color types of mink. The pelt production of these farms totaled 393,717 pelts. Forty-five (45) % of the ranches surveyed reported problems with nursing sickness, of which 87% encountered them every year. Thirteen (13) % of these farms had 10-40% of their females affected, 17% had an incidence rate of 5-9%, and 70% had less than 4% of the females with nursing sickness. Conclusions could not be made regarding color type and occurrence of the disease, as census responses did not differentiate these relationships. The onset of the disease was seen typically around 30-39 days (44%) or after 40 days (28%). Schneider and Hunter (1992) report typical onset of the disease at around 42 days after whelping. The farms reporting problems with nursing sickness had a higher average litter size per female on ranch at birth (5.2) in comparison to farms without nursing sickness (5.0). A trend toward higher average number of kits weaned was found on affected ranches (4.6) in comparison to those without nursing sickness (4.3, P=0.096) (Figure 1).

Figure 1. Average number of kits born versus average number of kits weaned for ranches with a history of nursing sickness (●) and those not experiencing problems (○). The regression equation for curve A (nursing sickness ranches) is $y = -12.83 + 6.19x - 0.54x^2$ (R^2=0.63), and for curve B (healthy ranches) $y = 2.74 + 0.05x + 0.05x^2$ (R^2=0.44).



These findings are similar to those of Clausen *et al.* (1992) who found that the total biomass of kits raised and weaned by dams developing nursing sickness was significantly larger than that of apparently healthy dams (5.4 versus 5.0 kits per litter).

Regarding ranch history (Table 1), farms reporting problems with nursing sickness more commonly housed mink in multi-row sheds (62%) than those not having problems (33%) (P=0.020). Shed orientation was also shown to differ between the two groups, with 25% of healthy ranches orientated East-West compared to 37% in the nursing sickness group (P=0.067).

Table 1. Summary of ranch history parameters surveyed for farms with a history of nursing sickness and those not experiencing problems (healthy).

Parameter		Nursing Sickness	Healthy	P-value
Kits born		5.2 ± 0.14	5.0 ± 0.19	0.335
Kits weaned		4.6 ± 0.09	4.3 ± 0.15	0.096
		% responses	% responses	
Shed type				0.020
	Two-row	28.6	44.4	
	Multi-row	61.9	33.3	
Shed orientation				0.067
	North-South	45.4	66.7	
	East-West	36.4	25.0	
Distance between sheds				0.072
	10-14 ft	57.1	55.6	
	15-19 ft	23.8	33.3	
	>20 ft	19.1	11.1	
Roofing material				0.171
	Fiberglass	4.8	11.8	
	Aluminum/steel	90.5	82.4	
	Other	4.8	5.9	
Watering system				0.052
	Water cups	28.6	44.4	
	Automatic nipple	23.8	16.7	
	Both	47.6	38.9	

Therefore, more southern exposure of the sheds on the farms with nursing sickness may have resulted in more heat stress in the females during lactation. Tauson (1998) has reported that prolonged periods of high ambient temperature may be hazardous for lactating mink decreasing energy intake and resulting in energy deficit and excessive mobilization of body reserves. Some farms reported that they experienced more problems in years when they had cold weather during the nursing season. Average reported temperatures throughout this phase and the rest of the production year, however, were not different between the two groups. Although seemingly contradictory, cold weather may increase the level of stress in the lactating female. The demand for more energy due to higher need for body temperature regulation may result in higher nutrient turnover and elevated heat production. Cold weather is also likely to keep the female in the nest longer with the kits. This would reduce her opportunity for exercise, would increase crowdedness as well as elevate the ambient temperature in the microclimate within the nest causing the female potentially to experience higher levels of (heat) stress.

In the survey, roofing material of sheds was not found to differ between groups, however differences were seen in distance between sheds (P=0.072). Healthy farms had more of their sheds spaced 15-19 ft a part, whereas farms with nursing sickness had their sheds more often either closer or further in distance from each other. This finding, although statistically meaningful, may be of minor practical importance. More importantly, the types of watering systems differed significantly between the healthy and nursing-sickness ranches (P=0.052). Forty-four (44) % of the healthy ranches provided water in cups only, whereas 29% of the farms reporting problems with nursing sickness used water cups as the sole means of providing water to the mink. The combination of both nipple drinkers and cups was used by 48% of nursing sickness ranches and by 39% of healthy farms whereas nipple drinkers only were employed by 24% and 17% of respondents, respectively. The method of provision and the availability of water appear to be

likely contributors to the development of nursing sickness and the associated dehydration in the mink female. It has previously been suggested that the water source together with ambient temperature and the number of kits contribute to the fluid deficit experienced by the lactating female (Schneider & Hunter, 1992). The manner in which water is provided to the female and her kits may have both behavioral and physiological consequences. The periodical filling of the water cups, either manually or automatically, perhaps acts as an audible stimulus encouraging the female and the kits to leave the nest more often and drink water. Practical observations by some ranches suggest that the kits are likely to learn the location of the water early, when using cups, especially if the cups are situated close to the nest box entrance. As a result, the amount of water consumed by the kits and the frequency of drinking by the female may be increased alleviating the fluid deficit.

Animal Management

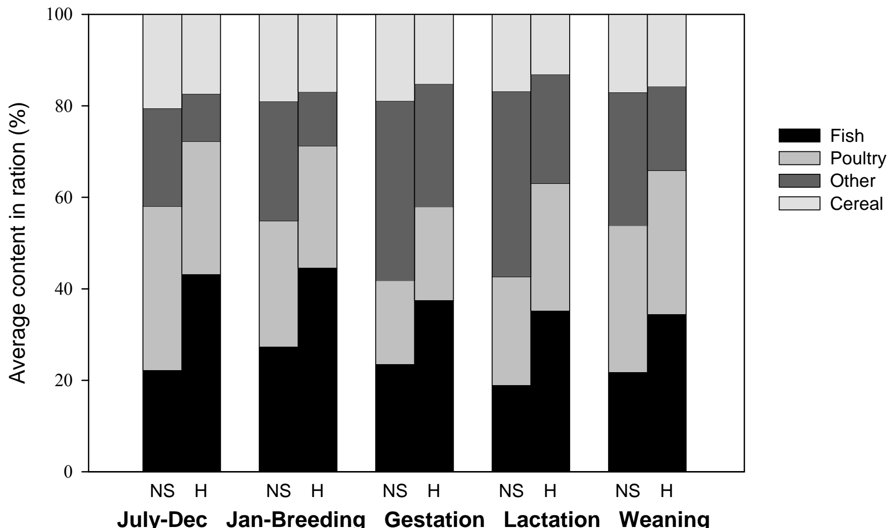
The main animal management practices surveyed among the healthy and affected ranches are presented in Table 2. On healthy farms, the main breeder female selection criteria used in November focused more on body length (healthy 83%, nursing-sickness 62%, P=0.091), whereas body weight was more emphasized on the farms with problems (healthy farms 17%, nursing-sickness farms 43%, P=0.062). Selection for body weight was also found to be more prevalent in February on ranches experiencing problems with the disorder (healthy 0%, nursing-sickness 29%, P=0.052). One hundred percent of ranches affected by nursing-sickness reported conditioning of breeder females, compared to 78% of healthy farms (P=0.037). Of the farms that practiced conditioning, 95% of those experiencing nursing sickness began conditioning between January and February, compared to 15% of healthy farms beginning between September and December, and 77% between January and February (P=0.065). Methods of conditioning also varied between the two groups (P=0.049). Eight (8) % of healthy farms tried to keep their females active, whereas none (0 %) of the nursing-sickness ranches employed this practice. On the healthy farms, reduced feed allowance was practiced by 69% and dietary fat content was reduced by 15% of the respondents, while 59% of the nursing-sickness farms reduced the amount of feed and 29% reduced the amount of fat. Both groups marked thin or obese females (healthy 8%, nursing-sickness 12%). It is apparent, that selection for body length is likely associated with a more lean body type, whereas selection for body weight may result in heavier body condition and thus more body fat. In carnivore companion animals, both body fat excess (obesity) and low physical activity are associated with poor glycemic regulation (Burkholder & Toll, 2000, Zicker et al., 2000). The farms selecting their breeder females based on body weight and not encouraging exercise may inadvertently impair the females’ ability to regulate blood sugar levels, causing disruption in glucose homeostasis (Børsting & Gade, 2000).

The age at which litters are weaned differed between the farms (P=0.001), with 10% weaning at 5-6 weeks, 50% at 7 weeks and 20% at 8 weeks on affected farms, compared to 20%, 20%, and 33% on healthy farms, respectively. The method of weaning and the amount of handling experienced by the females did not differ between the two groups. Although not found to a significant factor in the survey, the handling of the females should be minimized, as the time of weaning is known to be associated with elevated stress and cortisol levels in the mink female (Clausen et al., 1999). Handling of the females during this period of high stress is likely to cause further mobilization of nutrients from storage (Wamberg et al. 1992), augmenting the hyperglycemia and further compromising glycemic control. Handling also increases body core temperature, causing so called stress-induced hyperthermia in the mink (Korhonen et al. 2000), and can therefore exacerbate the female’s heat stress.

Table 2. Summary of animal management parameters surveyed for ranches with a history of nursing sickness and those not experiencing problems (healthy).

Parameter		Nursing Sickness	Healthy	P-value
Breeder selection criteria		% responses	% responses	
November				
	Reproduction	85.0	94.4	0.278
	Fur characteristics	85.7	83.3	0.333
	Selected litter mates	19.1	5.9	0.203
	Estimated pelt size	57.1	38.9	0.136
	Body length	61.9	83.3	0.091
	Body weight	42.9	16.7	0.062
	Health history	47.6	16.7	0.035
	Temperament	38.1	22.2	0.159
February				
	Reproduction	94.1	91.7	0.502
	Fur characteristics	88.2	83.3	0.378
	Selected litter mates	17.6	16.7	0.378
	Estimated pelt size	35.3	25.0	0.272
	Body length	41.2	58.3	0.199
	Body weight	29.4	0.0	0.052
	Health history	47.1	25.0	0.155
	Temperament	29.4	16.7	0.262
Conditioning of breeder females				0.037
	Practice conditioning	100.0	77.8	
Timing				0.065
	September - December	0.0	15.4	
	January- February	95.2	76.9	
Method				0.049
	Keep active	0.0	7.7	
	Reduce feed	58.9	69.2	
	Reduce fat	29.4	15.4	
	Mark thin/obese females	11.8	7.7	
Weaning of kits				0.001
	5-6 weeks	10.0	20.0	
	7 weeks	50.0	20.0	
	8 weeks	20.0	33.0	
Weaning method				0.111
	All kits at once	17.6	18.8	
	All kits but one	23.5	18.8	
	Remove dam	58.8	62.5	
Handling during nursing				0.120
	Handle female	47.4	38.9	

Figure 2. Average content of fish, poultry, other animal by-product ingredients and cereal in the diet throughout the production year for ranches with a history of nursing sickness (NS) and those not experiencing problems (healthy, H). The production year is divided into the following periods: July-December, January-breeding, gestation, lactation, and weaning.



Feed Composition and Feeding Management

The feed composition differed greatly between the farms. The farms without nursing sickness problems fed much more fish in their diet (34-42.5%) throughout the production year than the farms, which encountered problems (18-27%) (Figure 2). Through July to December (growing and furring) average fish content was found to be significantly higher in the apparently healthy farms (42.5%) than in the affected farms (22%)(P=0.049). Differences were also observed in the dietary content of other animal by-products (i.e. not fish or poultry) during this period (healthy 10%, nursing-sickness 21%, P=0.038). The amount of other animal by-products fed differed also at breeding (healthy 20%, nursing-sickness 35%, P=0.004), gestation (healthy 26%, nursing-sickness 38%, P=0.061), and lactation (healthy 23%, nursing-sickness 39%, P=0.040). The average content of cereal, fish, poultry and other animal by-products in the diet during the remaining phases of production were not found to differ between healthy and affected ranches. Dietary n-3 fatty acids, commonly found in fish, have been shown to improve glucose transport and metabolism resulting in improved glucose tolerance (Takahashi and Ide 2000). It is likely that the higher amount fish, providing more of the n-3 fatty acids, fed on the ranches not experiencing problems with nursing sickness may help the mink females to better regulate their blood sugar levels during lactation thus preventing the occurrence of the metabolic disorder of nursing sickness. Although the largest difference in the level of fish in the diet was observed during the fall months, the dietary background of the female will have long-lasting impacts influencing the composition of both body and milk fat during the nursing period. These results support the suggestion that a deficiency may develop in the lactating mink particularly during the latter part of the nursing period due to the substantial secretion of the n-3 fatty acids in the milk (Rouvinen-Watt 2003). The high physiological demand for the n-3 fatty acids during lactation may result in poorer glucose tolerance in females, which do not have an adequate dietary supply or have not accumulated adequate quantities of the n-3 fatty acids in their body fat reserves.

Additionally, it was suggested by some respondents that changing the location of the feed or water cups or providing solid false bottoms alleviated problems with nursing sickness. It is important to note, that these practices encourage the female and the kits to leave the nest box more often and the kits to start exploring their environment earlier. This increases the level of exercise by the female, helping with glucose clearance form the blood stream, and also alleviates crowdedness and heat stress in the nest environment.

The nursing sickness survey has identified several on-farm practices, related to breeder selection, animal management and feeding management, which promote better glycemic control in the lactating females. It is apparent that these practices are strongly associated with the reduced occurrence of nursing sickness.

Conclusions

According to a producer survey carried out in North America, mink ranches that select for good mothers with large litters experience more problems with nursing sickness. Crowdedness in the nest box may contribute to elevated stress levels on the females making the problem more severe. Genetic factors contribute to the incidence since selection for heavier body weight increased the occurrence while selection for body length reduced problems with nursing sickness. As handling may exacerbate the females' stress levels it should be minimized. Ranches that fed more fish experienced fewer problems with nursing sickness. Reduced nursing burden on the females either by selecting for optimum litter size or providing feed and water for the kits early may help alleviate problems. Encouraging the female to leave the nest box also appears to be helpful by increasing exercise, as well as reducing crowdedness and associated (heat) stress. Regarding breeder selection, animal management, and feeding management, several on farm practices were identified, which promoted better glycemic control in the lactating females. These practices appear to be strongly associated with the reduced occurrence of nursing sickness.

Acknowledgements

The Nursing Sickness Survey was supported by the Canada Mink Breeders Association, the Heger Company, and the Nova Scotia Fur Institute. We thank all the mink ranchers who participated in the survey for providing the valuable data for this research and sharing their practical knowledge.

References

Børsting, C.F. and Gade, A. 2000. Glucose homeostasis in mink (*Mustela vison*): A review based on interspecies comparisons. Scientifur 24, 9-18.

Burkholder, W. J. and Toll, D. W. 2000. Obesity. Pp. 401-430 in Small Animal Clinical Nutrition (Eds. Hand, M. S., Thatcher, C. D., Remillard, R. B. and Roudebush, P.). Mark Morris Institute. Walsworth Publishing Company Marceline, Missouri.

Clausen, T. N., Hansen, O. and Wamberg, S. 1999. Plasma cortisol concentration as an indicator of stress in mink dams at weaning. Scientifur 23, 271-273.

Clausen, T.N., Olesen, C.R., Hansen, O. and Wamberg, S. 1992. Nursing sickness in lactating mink (*Mustela vison*): I. Epidemiological and pathological observations. Can. J. Vet. Res. 56, 89-94.

Korhonen, H., Hansen, S. W., Malmkvist, J. and Houbak, B. 2000. Effect of capture, immobilization and handling on rectal temperatures of confident and fearful male mink. J. Anim. Breed. Gen. 117, 337-345.

Rouvinen-Watt, K. 2003. Nursing sickness in the mink - a metabolic mystery or a familiar foe? Review Article. Can. J. Vet. Res. 67, 161-168.

SAS Version 8. 1999. SAS Institute Inc., Cary, NC, USA.

Schneider, R.R. and Hunter, D.B. 1992. Nursing disease in the mink. Scientifur 16, 239-242.

Takahashi, Y. and Ide, T. 2000. Dietary n-3 fatty acids affect mRNA levels of brown adipose tissue uncoupling protein 1, and white adipose tissue leptin and glucose transporter 4 in the rat. Br. J. Nutr. 84, 175-184.

Tauson, A.-H. 1998. Water intake and excretion, urinary solute excretion and some stress indicators in mink (*Mustela vison*): effect of ambient temperature and quantitative water supply to lactating females. Br. J. Nutr. 80(6), 555-564.

Wamberg, S., Clausen, T.N., Olesen, C.R. and Hansen, O. 1992. Nursing sickness in lactating mink (*Mustela vison*): II. Pathophysiology and changes in body fluid composition. Can. J. Vet. Res. 56, 95-101.

Zicker, S. C., Ford, R. B., Nelson, R. W. and Kirk, C. A. 2000. Endocrine and lipid disorders. Pp. 850-885 in Small Animal Clinical Nutrition

(Eds. Hand, M. S., Thatcher, C. D., Remillard, R. B. and Roudebush, P.). Mark Morris Institute. Walsworth Publishing Company Marceline, Missouri.

II – 8 RP

Body condition and glycemic control in mink females during reproduction and lactation

A. M. Hynes[1], K. Rouvinen-Watt[1] and D. Armstrong[2]
[1]Canadian Center for Fur Animal Research, Nova Scotia Agricultural College, Department of Plant and Animal Sciences, Truro, Nova Scotia, Canada [2]Heger Company, North St. Paul, Minnesota, USA

Abstract
A two-part diagnostic pilot study was conducted. Firstly, 98 breeder females were weighed and scored for body condition at breeding, late gestation, and mid and late lactation. The mink were tested for urine parameters at the above time points and blood glucose before and after weaning. Glucosuria was found to be present in the mink females at all stages of the reproductive cycle. There was a negative relationship between body weight and blood glucose levels late in the nursing period. One week after weaning most females were normoglycemic indicating that the hyperglycemia observed was transitory. Secondly, 518 adult and juvenile mink females of black and brown color types were scored for body condition and tested for urine glucose prior to breeding, late gestation, and around the time of weaning. Adult females and the brown color type mink were generally in much heavier body condition throughout the reproductive season. Results indicate that a varying percentage of mink breeder females exhibit glucosuria and that the occurrence may be related to the body condition of the females. The diagnostic findings of hyperglycemia and glucosuria in the mink females during the reproductive cycle indicate impaired glycemic control. Further investigation of these parameters as causative factors to the development of nursing sickness is warranted.

Introduction
In the newly proposed hypothesis for the etiology of nursing sickness in the mink (*Mustela vison*) (Rouvinen-Watt, 2003), striking similarity is suggested between the clinical symptoms seen in the affected mink dams and those observed in the metabolic syndrome associated with acquired insulin resistance. Type 2 diabetes, or non-insulin dependent diabetes mellitus (NIDDM) is a disorder characterized by insulin resistance or abnormal insulin secretion (Zimmet *et al.,* 2001), with hyperglycemia as a dominant feature (Palumbo, 2001). In states associated with marked insulin resistance, the ability of insulin to stimulate the translocation of GLUT-4, the insulin-responsive glucose transporter, and therefore glucose uptake, in muscle and adipose cells is abnormally diminished (Khayat *et al.,* 2002). Although not documented in mink, various animal and human models exhibit a varying degree of pathophysiology related to type 2 diabetes (Wood and Trayhurn, 2003). Such has been reported in another member of the *Mustelidae* family, a black-footed ferret that exhibited, among other symptoms, weight loss and hyperglycemia (Fox and Marini, 1998). The presence of glucose in the urine, or glucosuria, is an indication of the animal's poor ability to regulate blood sugar levels over a period of time and is a key finding in untreated diabetic dogs and cats (Hoenig, 2002). Major abnormalities found in feline diabetes include impaired insulin secretion, peripheral insulin resistance and increased basal hepatic glucose production (Behrend, 2002a). Obesity has been identified as a risk factor for the development of diabetes in both cats and dogs (Hoenig, 2002). The objective of this two-part study was to investigate glucose regulation with relation to body condition in female mink during the reproduction and lactation periods.

Materials and Methods
A two-part diagnostic pilot study was conducted. The body condition scoring system, which is outlined in Appendix A, was developed to assist in evaluating the amount of body fat and lean body mass in the mink independently of the body weight of the mink. Data was analyzed using Fisher's exact test and Proc GLM in SAS (1999) and regression analyses were done in SigmaPlot. Statistical significance was set at $P<0.05$.

Part 1: ***Urine and blood glucose testing of CCFAR mink herd***

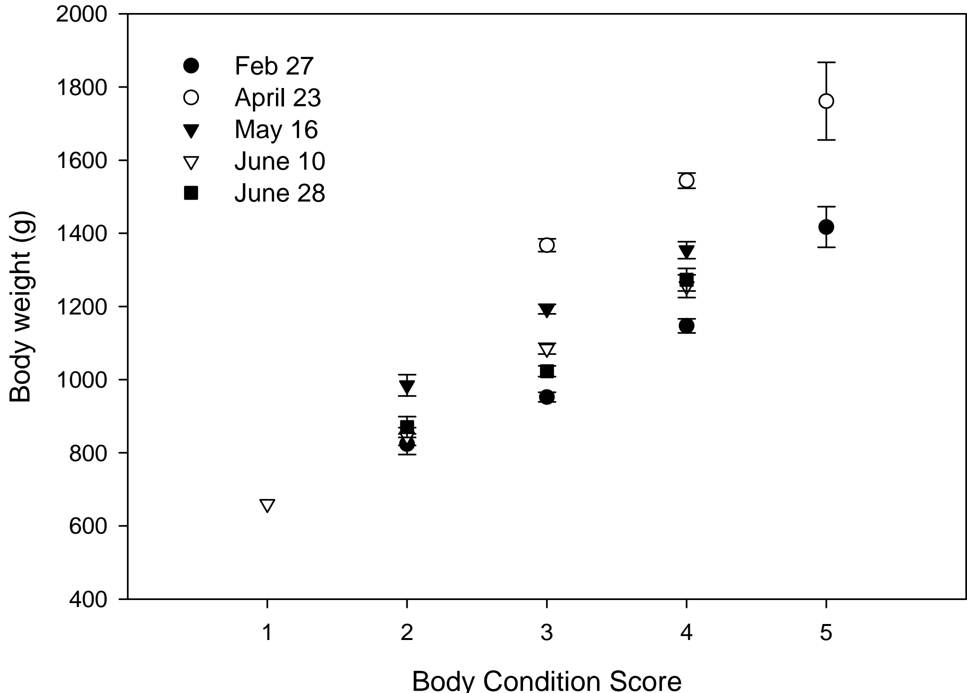
Materials and Methods

The mink herd (98 breeder females) at the Canadian Center for Fur Animal Research at the Nova Scotia Agricultural College was scored for body condition (BCS) and then weighed in February (breeding), April (late gestation), May (mid-lactation), and June (late lactation and after weaning) of 2002. Upon handling, voluntarily voided urine was collected from the females and these samples were tested for urine parameters, including glucose (DiaScreen 10 test strips, MEDgenesis, MN) at all the above time points. In addition, on June 10 (late lactation) and June 28 (1 wk after weaning) a post-prandial blood sample, drawn from a clipped toenail, was analyzed for glucose concentration using an Accu-Chek™ Compact blood glucose monitor (Roche Diagnostics, Laval, Quebec).

Results and Discussion

The body condition scoring system developed for this study was found to be a useful and practical tool for assessing the degree of obesity in the mink. A high correlation between BCS and body weight was observed at all stages (Figure 1). Overall mean weight was found to be marginally different between score 1 (very thin) (715.2±115.8g) and 2 (thin) (933.0±17.5g) (P=0.06), however significant differences (P<0.001) were observed between all other categories; 3 (ideal) (1121.1±6.6g), 4 (heavy) (1310.8±11.1g) and 5 (obese) (1555.2±52.0g). These differences also remained distinguishable (P<0.001) at the different stages of the reproductive cycle although the body weight of the females varied greatly, being the heaviest during late gestation (1367.8±28.0g) and the lowest at breeding (965.6±27.5g). A marginal difference was observed between body weights at late lactation and after weaning (P=0.06).

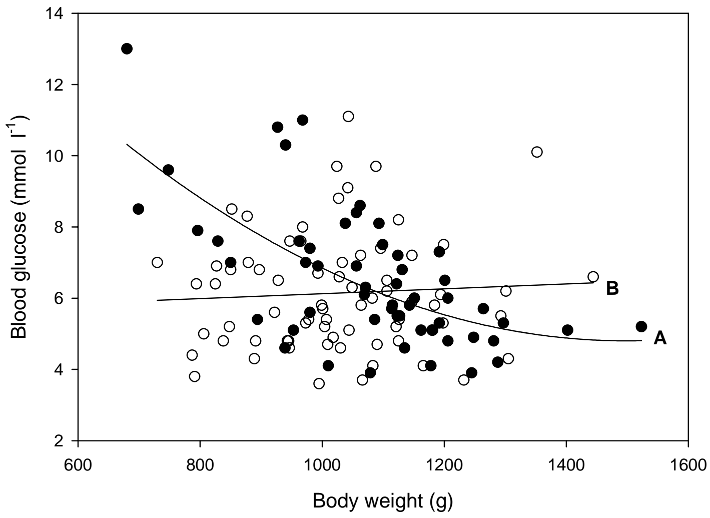
Figure 1. Relationship between body condition score and body weight of mink breeder females from breeding (late February) until one week after weaning (June 28). The regression equation for February is y = 393.4 + 197.6x (R^2=0.98), April: y = 769.7 + 196.9x (R^2=0.99), May: y = 622.7 + 184.8x (R^2=0.99), June 10: y = 454.4 + 202.7x (R^2=0.99), and June 28: y = 451.4 + 201.4x (R^2=0.98).



Glucosuria was found to be present in the mink females at all stages of the reproductive cycle, with 24.2% of collected samples showing glucose at breeding, 20.8% at late gestation, 10.5% at mid-lactation, 27.0% at late lactation sampling and 12.3% after weaning. The values detected were either 50 or 100 mg dl^{-1} with the exception of a female, which died after exhibiting the typical symptoms of nursing sickness. Her urine glucose was measured at 1000 mg dl^{-1} at the time of death. The excretion of glucose in the urine of the mink dams demonstrates that their renal absorptive threshold has been exceeded. The presence of glucosuria at each time point indicates that the inability to regulate blood glucose may be a preexisting condition in the mink dams. Body weights did not significantly differ between those showing glucosuria (1229.4±18.8g) and not (1228.7±15.2g). Significant differences ($P<0.001$) were found in the occurrence of urine glucose within the scoring categories; 33.3% of the thin (2), 16.1% of the ideal, 18.4% of the heavy and 40.0% of the obese females showed glucosuria. These findings indicate that independent of body weight, females in non-ideal condition, in particular those scored as thin or obese, may have a propensity for poor glucose regulation. Both the excess and the lack of adipose tissue, the main buffer for the daily influx of dietary nutrients, have been shown to interfere with insulin-mediated glucose disposal (Frayn, 2001).

The measurement of blood glucose levels of the females in relation to body weight in late lactation and after weaning indicated that there was a negative relationship between body weight and blood glucose levels late in the nursing period (Figure 2) ($P<0.001$). However, no relationship was observed between these factors after weaning, indicating that while under the stress of nursing, underweight females may not have the ability to regulate glucose and that once the lactation demand is removed the dams are able to reestablish homeostasis. Blood glucose levels were not found to be significantly different between body condition categories 1 (9.7 mmol l^{-1}) and 2 (7.7±0.4 mmol l^{-1}), however significant differences ($P<0.05$) were observed in all other comparisons respectively (BCS 3, 6.2±0.2 mmol l^{-1} and BCS 4, 5.4±0.4 mmol l^{-1}). In relation to both body weight and body condition, the smallest females were shown to have the highest blood sugar levels. Along with obesity, lipodystrophy, or the deficiency of adipose tissue, has been identified as an accompanying factor in the development of insulin resistance and type 2 diabetes (Frayn, 2001). The absence of adipose cells in an under conditioned nursing female may result in the accumulation of fat in glucose metabolizing tissues, inducing insulin resistance and effectively disrupting peripheral glucose disposal.

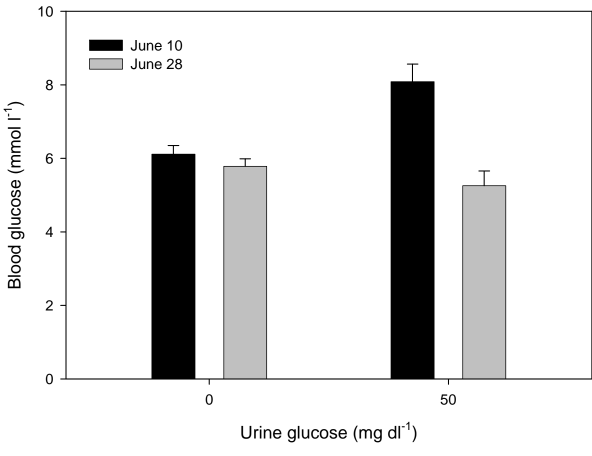
Figure 2. Relationship between body weight and blood glucose concentration of mink breeder females at late lactation (June 10) (●) and after weaning (June 28) (○). The regression equation for curve A (before weaning) is $y= 23.30 - 0.02x + 0.00000824x^2$ ($R^2=0.40$), and for line B (after weaning) $y = 5.44 + 0.00069x$ ($R^2=0.00374$).



A significant number of the females showed hyperglycemia in late lactation (6.8±0.2 mmol l^{-1}), whereas this was reduced after weaning of the litters (5.9±0.2 mmol l^{-1}) (P=0.002). One week after weaning the blood sugar concentration of most of the females had returned to normal levels. Wamberg *et al.* (1992) have reported mean glucose levels of 5.3∀0.3 mmol l^{-1} for apparently healthy lactating females. This is an indication that the hyperglycemia observed in the females is a transitory condition associated with late lactation and is largely reversed after the stresses of the nursing and weaning of the litters have been eliminated.

The urine glucose concentrations of the females in late lactation were shown to be strongly dependent on the blood glucose concentrations (P<0.001). Females with measurable quantities of glucose in their urine had an average blood sugar level of 7.8± 0.4 mmol l^{-1}, whereas females with no glucosuria had a mean blood sugar level of 6.2 ± 0.2 mmol l^{-1} (Figure 3). After weaning, no difference was observed between the blood sugar levels of the females with (5.2 ± 0.35 mmol l^{-1}) and without glucosuria (5.7± 0.18 mmol l^{-1}). Behrend (2002b) identifies increased excretion of glucose as a cause of polyuria and increased obligatory water loss. With the increased occurrence of glucose in the urine during late lactation, nursing females are at higher risk for both dehydration and energy loss, factors highly associated with the development of nursing sickness.

Figure 3. Urine glucose concentration in relation to blood glucose concentration of mink breeder females before (June 10) and after weaning (June 28).



Part 2: *Urinalysis field study of mink breeder females*

Materials and Methods

During the winter, spring and summer of 2002, urinalyses were conducted on six collaborating mink ranches to examine glucose regulation in mink females during the different stages of the reproductive cycle. Three farms with black type mink and three farms housing the brown color type were used. Both juvenile and adult females per ranch were scored for body condition according to criteria presented in Appendix A, and voluntarily voided urine was analyzed for glucose using DiaScreen 1G test strips (MEDgenesis, MN). A total of 518 mink females started on the study with a total of 1320 urinalysis tests being performed prior to breeding (early February), late gestation (mid-April), and around the time of weaning (late June). It is to be noted that the records provided did not allow for tracking of individual animals and that not all females could be tested at each time point due to difficulty in securing a urine sample. The results of the urinalysis field study are therefore to be considered more qualitative than quantitative.

Results and Discussion

Obesity is characterized by the increased storage of fat in adipose cells that, in turn, causes them to fail in their normal role of protecting other tissues, i.e. skeletal muscle, liver and pancreatic beta cells, from the daily influx of dietary fatty acids (Frayn, 2001). This build up leads to impaired glucose metabolism (Frayn, 2001). Overall, results throughout the reproductive cycle (Table 1) show a significantly higher percentage (P<0.001) of adult females in heavy or obese body condition (11.9-52.1%) compared to juvenile females (9.8-35.8%), with the exception of the black type females at weaning where findings were similar. Increasing age of the lactating dam has been indicated as a major determinant in the development of nursing sickness (Clausen et al., 1992). This may be influenced by two factors; firstly, circulating non-esterified fatty acids, the levels of which are increased in overweight and obese individuals, are known to be potent stimulants for hepatic glucose production (Frayn, 2001). Secondly, the older females have larger litters and therefore a higher demand for milk production, the demands of which are largely met by hepatic gluconeogenesis

(Børsting and Gade, 2000). Fink and Børsting (2002) have suggested that uncontrollable gluconeogenesis causes hyperglycemia in the female mink during lactation. Therefore, the older mink dams may be more prone to poor glycemic regulation due to their higher demands for hepatic glucose production in support of the higher milk production as well as increased hepatic glucose output caused by their heavier body condition.

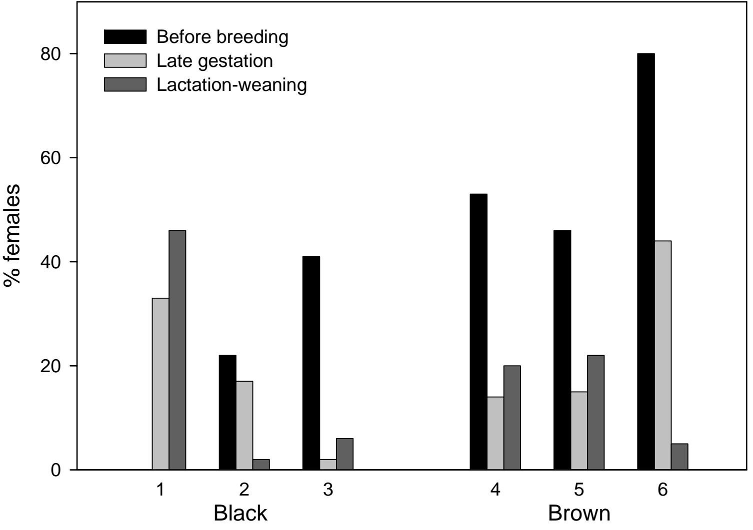
It should be noted that the percent of glucosuria observed during these periods was comparable between the age groups (6.7-38.2% adult, 7.1-35.8% juvenile). A significant difference in urine glucose output was observed only in the brown type females prior to breeding (56.3% adult, 51.2% juvenile, P=0.013). The similarities observed in the percentages of both adult and kit females showing glucosuria, despite the higher percent of over-conditioned adults, may be a result of prior culling of older females with impaired glucose regulation. A confounding factor may be that samples of urine may not have been obtained from dehydrated animals with severely compromised glycemic control; the number of procured samples dropped from 518, prior to breeding, to close to 400 at subsequent sampling periods.

The brown color type mink were generally in much heavier body condition than the black type mink throughout the reproductive season (P<0.001) (Figure 4). This indicates ranch level differences among the genetic factors and/or animal husbandry practices. Throughout reproduction and lactation urine glucose values between 50 and 500 mg dl^{-1} were detected in the black type females, whereas the brown type showed sugar in the urine more frequently (5.5-16.4% black, 7.9-53.9% brown, P<0.001) and at higher levels, with values up to 1000 mg dl^{-1} being detected. The higher occurrence of over-conditioned brown type dams, in combination with the higher incidence of glucosuria, points toward a positive association between the two. Overall, the incidence of obesity and the presence of sugar in the urine, observed in each age and colour group tested, indicate that body condition may have a significant impact on the mink dam’s ability to maintain glucose homeostasis throughout the reproductive cycle. Further investigation is needed into their role in the pathology of nursing sickness.

Table 1. Percentage of mink breeder females in heavy and obese body condition and the percentage of females showing glucose in the urine before breeding, during late gestation and around weaning. For body condition scoring, see Appendix A.

		Time period					
Color type		**Before breeding**		**Late gestation**		**Lactation-weaning**	
Initial Age	# tested	% heavy	% glucosuria	% heavy	% glucosuria	% heavy	% glucosuria
Black							
juvenile	124	21.2	16.1	11.9	6.0	5.5	15.5
adult	125	42.0	17.6	20.2	5.2	5.3	6.3
Total Black	249	31.7	16.4	16.4	5.5	5.4	10.2
Brown							
juvenile	127	54.3	51.2	18.1	7.9	12.5	10.3
adult	142	68.3	56.3	33.8	8.0	16.9	9.7
Total brown	269	61.7	53.9	26.1	7.9	14.8	10.0
All mink							
juvenile	251	35.8	33.9	15.6	7.1	9.8	15.5
adult	267	52.1	38.2	27.9	6.7	11.9	8.2
Total	518	44.2	36.1	22.0	6.9	10.9	10.0

Figure 4. Percentage of mink breeder females in heavy and obese body condition by color type and ranch before breeding, during late gestation and around weaning. Black type mink: ranches 1-3; Brown type mink: ranches 4-6. For body condition scoring, see Appendix A.



Conclusion

The body condition scoring system developed for this study was found to be a useful and practical tool for assessing the degree of obesity in the mink. The results of the diagnostic testing pilot study indicate that a varying percentage of mink breeder females have high blood sugar levels and sugar in their urine and that the occurrence of this is very likely related to the body condition of the females. In other species, these diagnostic findings are associated with obesity, and the acquired insulin resistance syndrome, also known as type 2 diabetes. The large differences observed between the individual farms indicate that this may be dependent on the genetic background of the mink or the animal management practices used on the ranch, or a combination of the two. The diagnostic findings of hyperglycemia and glucosuria in the mink females during the reproductive cycle clearly indicate impaired glycemic control. Further investigation of these parameters as causative factors to the development of nursing sickness is warranted.

Acknowledgements

This research was supported by the Heger Company and the Canada Mink Breeders Association. We thank the collaborating mink ranchers for their time and contribution to this project. Without their participation this research would not have been possible.

References

Behrend, E.N. 2002a. Diabetes mellitus: an update on oral hypoglycemic agents and monitoring agents. Vet. Med. 97 (10), 743-751.

Behrend, E.N. 2002b. Managing diabetes insipidus and other causes of polyuria and polydipsia. Vet. Med. 97 (10), 753-761.

Børsting, C. F. and Gade, A. 2000.Glucose homeostasis in mink (*Mustela vison*). A review based on interspecies comparisons. Original Review. Scientifur 24, 9-18.

Clausen, T.N., Olesen, C.R., Hansen, O., and Wamberg, S. 1992. Nursing Sickness in the lactating mink (*Mustela vison*). I. Epidemiological and pathological observations. Can. J. Vet. Res. 56, 89-94.

Fink, R. and Børsting, C. F. 2002. Quantitative glucose metabolism in lactating mink (*Mustela vison*) – effects of dietary levels of protein, fat and carbohydrates. Acta Agric. Scand., Sect. A. Anim. Sci. 52, 34-42.

Fox, J.G. and Marini, R.P. 1998. Diseases of the endocrine system. Pp. 301 in: Fox, J.G. (Ed.) Biology and Diseases of the Ferret, 2nd ed. Williams & Wilkins, Baltimore.

Frayn, K.N. 2001. Adipose tissue and the insulin resistance syndrome. Proc. Nutr. Soc. 60, 375-380.

Hoenig, M. 2002. Comparative aspects of diabetes mellitus in dogs and cats. Mol. Cell. Endocrinol. 197, 221-229

Khayat, Z.A., Patel, N., and Klip, A. 2002. Exercise and insulin stimulated muscle glucose transport: Distinct mechanisms of regulation. Can. J. Appl. Physiol. 27, 129-151.

Rouvinen-Watt, K. (2003). Nursing sickness in the mink – a metabolic mystery or a familiar foe? Can. J. Vet. Res. 67, 161-168.

SAS Version 8. 1999. SAS Institute Inc., Cary, NC, USA.

Wamberg, S., Clausen, T.N., Olesen, C.R. and Hansen, O. 1992. Nursing sickness in lactating mink (*Mustela vison*): II. Pathophysiology and changes in body fluid composition. Can. J. Vet. Res. 56, 95-101.

Wood, I.S. and Trayhurn, P. 2003. Glucose transporters (GLUT and SGLT): expanded families of sugar transport proteins. Brit. J. Nutr. 89, 3-9.

Zimmet, P., Alberti, K.G.M.M. and Shaw, J. 2001. Global and societal implications of the diabetes epidemic. Nature 414, 782-787.

Appendix A. Body Condition Scoring System

Canadian Center for Fur Animal Research
Nova Scotia Agricultural College

BODY CONDITION SCORING OF MINK
USING A FIVE-POINT SCALE

SCORE 1. Very thin

- **The mink has an emaciated appearance with decreased muscle mass.**
- **The animal has a thin neck and a clearly V-shaped body.**
- **There is no body fat and the stomach is sunk in.**
- **Shoulder and hip bones can be seen and the ribs are easily felt.**

SCORE 2. Thin

- **The mink has a thin neck and a V-shaped waistline.**
- **There is no subcutaneous body fat layer.**
- **The shoulder and hip bones and the ribs can be easily felt**

SCORE 3. Ideal

- **The mink has a slender neck and a straight body shape.**
- **There is a slight amount of subcutaneous body fat.**
- **The shoulder and hip bones and the ribs can be easily felt.**

SCORE 4. Heavy

- **The mink has a thicker neck and a pear-shaped body.**
- **The ribs are difficult to feel.**
- **The shoulder and hip bones are covered by a moderate fat layer.**
- **An abdominal fat pad is present.**

SCORE 5. Obese

- **The mink has a thick neck with a slight brisket and a full body shape.**
- **The ribs are very difficult to feel.**
- **The shoulder and hip bones are covered by a moderate to thick fat layer.**
- **A fat pad is present in the abdomen and the tail.**
- **Fat deposits can be seen in the limbs and the face.**

Developed by Kirsti Rouvinen-Watt and Dean Armstrong
Technical assistance by Rick Russell and Rae MacInnis
January 21, 2002. Modified August 26, 2002

II – 10 RP

Wet kits in mink, a review

Clausen, T.N.[1] & Dietz, H.H.[2]
1. Danish Fur Breeders Research Centre, 112 C Herningvej, Tvis, DK-7500 Holstebro, Denmark; *tove.clausen@pfr.dk*
2. Danish Institute of Food and Veterinary Research, Dept. of Poultry, Fish and Fur Bearing Animals, Section for Pathology, 2 Hangovej, DK-8200 Aarhus N, Denmark; *hhd@dfvf.dk*

Summary
"Wet kits" (also known as " "greasy kits" or " sticky kits") in mink is a multifactorial disease in the lactation period with few known definitive releasing factors. The disease is known in all mink-producing countries in the northern hemisphere, and has been observed on commercial mink farms in Denmark for more than 40 Years (Svennekjær, 1954).
The definition of "wet kits" is when mink kits develop a greasy, sticky exudate on the skin surface especially in the neck, and tail, as well as on the claws, a red and swollen perianal region, frequently a yellowish-white diarrhoea and invariably a mewing, distressed behaviour.
The effects of bacteria, virus, management, feed, immunology of the animals and environmental factors on "wet kits" are discussed.
A lack of consistency in pathogenicity of bacteria and viruses isolated from wet kits and non-wet kits complicates experimental investigations.
An infectious etiology similar to diarrhoea in newborn calves and pigs has been postulated.
In calf and piglet diarrhoea Radostits et al. (1994) concluded that there is not a single etiology, but rather a complex interaction between enteropathogenic bacteria and viruses, other pathogens such as protozoa, the immunity of the animals, and the effects of the environment. With the addition of management factors to this list, the same theory might be valid for the etiology of "wet kits" in mink.
The recent finding of an astrovirus in diseased mink kits indicates that this virus may be one of the more important triggering factors in the wet kit syndrome.

Introduction
"Wet kits" is a problem of great economical importance for mink breeders in Denmark. The number of farms affected annually varies considerably and can be very high. The morbidity rate can vary from 0 to more than 30 percent, and the mortality is normally one to two kits per litter. Apart from the loss of kits a lot of time is spent on treatment and the medication can be rather expensive. Kits surviving the disease have a lower weight at weaning than unaffected kits, but the same weight and skin length at pelting (unpubl. obs).
A number of eliciting factors have been tested in Denmark in usually unpublished investigations. It has turned out to be very difficult to perform prospective as well as experimental studies of "wet kits" due to the high variation in annual morbidity rate. Furthermore, there is no obvious pattern in disease outbreaks among farms.

Bacteria
Bacteriological examinations of wet kits showed predominantly *Staphylococcus spp.* in kits up to two weeks of age and *E.coli* in older kits (Rattenborg et al., 1995). Various *E. coli* serotypes have been detected but no difference in serotypes or presence of virulence factors between healthy and diseased kits was found (Jørgensen et al., 1996; Vulfson et al, 2000). Most of the isolated bacteria were not considered to be primary enteric pathogens, but rather common opportunistic organisms (Schneider and Hunter, 1993; Jørgensen et al., 1996). However, weak symptoms of "wet kits" can be provoked by oral inoculation with *E. coli* and *Staphylococcus spp.* (Henriksen, personal communication). A similar phenomenon has been described in e.g. calves where the same virus and bacteria can be isolated from healthy as well as diseased calves though usually in a higher concentration in diseased animals (Radostits et al., 1994).
No clear epidemiological evidence of an infection spreading on the farm during outbreaks of wet kits has been found so far (Chriél et al. 1997).

Virus
An "atypical" rotavirus causing diarrhoea in 2 to 6-week-old ferret kits was isolated by Torres-Medina

(1987). Svansson (1991) found that intraperitoneal inoculation of a reovirus-like virus could cause symptoms resembling “wet kits”, and Järplid and Meyerland (1998) found histological alterations in the intestinal villus epithelium of wet kits resembling changes caused by rotavirus infection in neonatal calves.
Englund et al. (2002) found that astrovirus was a significant risk factor in the development of pre-weaning diarrhoea. Other factors, i.e. low body weight, coccoid bacteria adherent to enteric villi, and the presence of calicivirus, were also shown to increase the risk of pre-weaning diarrhoea.

Management
In mink production management problems as a contributing factor in the development of “wet kits” has not been very well investigated so far.
A questionnaire revealed that there were bigger problems on large farms in Denmark (Olesen & Clausen 1990), probably due to a high population density. It has also been shown that the frequency of wet kits in groups with an empty cage between the females was significantly lower than in groups with a female in each cage (Overgård, 2000).
Many other factors have been discussed as contributing to the onset of the disease. Stressing the females by feeding on top of the nest box early in the lactation period, too much handling of the animals, hot weather that causes the females to leave the kits, inadequate sanitation and hygiene in the nest box etc. are potential factors.
Flushing is often applied to female mink prior to breeding (Atkinson, 1996). Some mink farmers, however, tend to reduce the energy intake too much and an epidemiological examination of large data sets have shown that a low energy intake in late April predisposes to “wet kits” (Chriél, 1997).

Feed
Although feed has been incriminated on numerous occasions, several years of investigations into feed composition, the different raw materials etc. has not shown a clear connection between feed composition and outbreaks of “wet kits”. The only positive correlation between feed and “wet kits” is that very high amounts of fat in the feed during the lactation period can increase the frequency of “wet kits” (Olesen & Clausen, 1992).
The quality of the feed most probably will also be a contributing factor, but is not easy to prove.

Animals
Hunter & Schneider (1996) postulated that “wet kits” or adenitis of the neonatal cervical gland occurs frequently in mutation colour phases of mink. Our experience is that all colour types are affected but the most serious outbreaks are usually seen in black mink and blue mink.
Litters with many kits from young females giving birth late in the period are at greatest risk (Olesen & Clausen, 1990). So far, inheritance has not been shown as an important factor.
A few days after birth, the kits have the same amounts of antibodies against virus enteritis (parvovirus) in the blood as the female, independent of the number of kits in the litter (Uttenthal et al., 1999). The amount of antibodies in serum and colostrum in heifers is lower than in cows (Radostits et al. (1994), and if this is the same for young female mink, it may contribute towards making their kits more susceptible to the disease.
Kits born late will be disposed to a greater amount of infectious agents than kits born early in the period, thereby increasing the risk of “wet kits” for late born litters.

Mastitis
Mastitis in the females has been hypothesised as a triggering factor (Trautwein & Helmholdt, 1966, Henriksen, 1988)) although classical clinical signs of mastitis (rubor, dolor et calor) are rarely seen in Danish, lactating mink bitches. Investigations by Clausen & Dietz (2000) showed that mastitis is not a contributing factor in the wet kit syndrome in mink.

Milk
Insufficient milk production has also been suggested as a triggering factor, but the fact that most necropsied mink kits with diarrhoea had coagulated milk in their gastrointestinal tract (Henriksen, 1987; Dietz unpublished observations), and that palpation of the mammary glands of mink females with wet kits shows that there is a lot of milk (Clausen unpublished observations), proves it to be unlikely.
Changes in the milk as a triggering factor have been discussed and many investigations on mink milk composition have been carried out (Andersen et al., 2000; Bjergegaard et al., 1998; Bjergegaard et al., 1999; Bjergegaard et al., 2000; Bjergegaard et al., 2002; Clausen & Olesen, 1992; Clausen et al., 1998; Olesen et al., 1992; Wamberg et al., 1992). So far, no clear difference between milk from females with

wet kits and females with healthy kits has been shown.

Conclusion

A lack of consistency in pathogenicity of bacteria and viruses isolated from wet kits and non-wet kits complicates experimental investigations

An infectious etiology similar to diarrhoea in newborn calves and pigs has been postulated.

Radostits et al. (1994) concluded that there is not a single etiology of calf and piglet diarrhea but rather a complex interplay between enteropathogenic bacteria and viruses, other pathogens such as protozoa, the immunity of the animals, and the effects of the environment. With the addition of management factors to this list, the same theory might be valid for the etiology of “wet kits” in mink.

However, the recent finding of an astrovirus in diseased mink kits indicates that this virus may be one of the more important triggering factors in the wet kit syndrome.

Literature

Andersen, K.E., Bjergegaard, C., Buskov, S., Clausen, T.N., Mortensen, K., Sørensen, H., Sørensen, J.C., Sørensen, S., 2000. Glycosider i minkmælk (Glycosides in mink milk) Faglig Årsberetning for Dansk Pelsdyravlerforening. 1999, 155-162.

Anon. 1986. European Convention for the Protection of Vertebrate Animals used for Experimental and other Scientific Purposes. European Treaty Series No. 123. Council of Europe, Strasbourg 1986.

Atkinson, J., 1996. Mink nutrition and feeding. In: Mink, biology, health and disease. Eds.: Hunter, D.B. & Lemieux, N. Graphic and print services, University of Guelph, Ontario.

Bjergegaard, C., Clausen, T.N., Mortensen, K., Sørensen, H., 1998. Variationer af fedtindhold i minkmælk bestemt ved Superkritisk Ekstraktion (SFE) (Variations in mink milk fat content analysed by Supercritic Extraction). Faglig Årsberetning for Pelsdyrerhvervets Forsøgs- og Rådgivningsvirksomhed 1997, 221-227.

Bjergegaard, C., Clausen, T.N., Mortensen, K., Sørensen, H., Sørensen, J.C., Sørensen, S., 2000. Undersøgelse af proteiner og peptider i minkmælk. (Investigations of proteins and peptides in mink milk). Faglig Årsberetning for Pelsdyrerhvervets Forsøgs- og Rådgivningsvirksomhed 1999, 145-153.

Bjergegaard, C., Clausen, T.N., Dietz, H.H., Mortensen, K., Sørensen, H., Sørensen, J.C., 2002. Højt indhold af etoxyquin i foderet til minktæver i reproduktions- og diegivningsperioden samt minkhvalpe i vækstperioden. (High content of etoxyquin in feed for mink females in the reproduction and nursing periods and mink kits in the growing- furring period) Faglig Årsberetning for Pelsdyrerhvervets Forsøgs- og Rådgivningsvirksomhed 2001, 133-139.

Bjergegaard, C., Buskov, S., Clausen, T.N., Mortensen, K., Olsen, C.E., Sørensen, H., Sørensen, J.C., Sørensen, S., 1999. Mink milk composition important for optimal development of mink kits, determination of amounts and structure of individual compounds. In: NJF-Seminar, no. 308. Island 1999.

Chriél, M., Rattenborg, E., Hejlesen, C. & Møller, S.H., 1997. Undersøgelse af epidemisk forløb ved udbrud af fedtede hvalpe. (An investigation of the epidemiology of outbreaks of greasy kits). Dansk Pelsdyravl,60,232-233.

Chriél, M., 1997. Lad minktæverne selv bestemme. (Let the mink females decide them selves) Dansk Pelsdyravl,60,196-198.

Clausen, T.N. & Dietz, H.H., 2000. Mastitis in the lactating mink female *(Mustela vison S.)* and the development of “greasy kits”. Acta Vet. Scand., 41, 243-247.

Clausen, T.N., Olesen, C.R., 1992. Malkning af tæver med forskellig kuldstørrelse. (Milking of females with different litter sizes) Faglig Årsberetning 1991, 210-216.

Clausen, T.N., Mortensen, K., Sørensen, H., Sørensen, J.C., Sørensen, S., 1998. Anvendelse af IsoPrime til karakterisering af proteinsammensætning i minkmælk. (The use of IsoPrime for characterisation of the protein fractions in mink milk) Faglig Årsberetning for Pelsdyrerhvervets Forsøgs- og Rådgivningsvirksomhed 1997, 213-220.

Englund, L., Chriél, M., Dietz, H.H., Hedlund, K.O., 2002. Astrovirus epidemiologically linked to pre-weaning diarrhoea in mink. Vet. Micr. 85, 1–11.

Henriksen, P.: “Wet mink kits” - acute enteritis in pre-weaning mink. Proc. IV Int.congress in

fur animal prod. Eds.: B.D. Murphy & D.B. Hunter, Ontario, Canada,208-212,1988.

Hunter, D.B. & Schneider, R., 1996: Infertility and neonatal mortality. In: Mink, biology, health and disease. Eds.: Hunter, D.B. & Lemieux, N. Graphic and print services, University of Guelph, Ontario.

Järplid, B., Mejerland, T., 1998. Villous atrophy in the small intestine of mink kits with diarrhoea. Scientifur, 22, 2, 157-162.

Jørgensen, M., Scheutz, F., Strandbygaard, B., 1996. *Escherichia coli* and Virus isolated from "sticky" kits. Acta Vet.Scand.37, 163-169.

Henriksen, P., 1998. Personal communication.

Olesen, C.R., Clausen, T., 1992. Betydningen af diegivningsfoderets næringsstofsammensætning for minkhvalpes vækst, frekvensen af fedtede hvalpe og tævens huld. (The importance of feed composition during lactation for the growth, frequency of greasy kits and body condition of the females). Faglig Årsberetning for Dansk Pelsdyravlerforening.1991,217-225.

Olesen, C.R., Clausen, T., 1990. Fedtede hvalpe. Resultater fra Forsøgsfarmen Vest 1989. (Greasy kits. Results from the experimental farm 1989). Faglig Årsberetning for Dansk Pelsdyravlerforening.1989, 154-168.

Olesen, C.R., Clausen, T.N., Wamberg,S., 1992. Compositional changes in mink (*Mustela vison*) milk during lactation. Norwegian J. Agr. Sci., Suppl. No. 9, 308-314.

Overgård, L., 2000. Effekt af tomt bur mellem minktæver i reproduktionsperioden. (Effect of an empty cage between mink females in the reproduction period) Fagllig Årsberetning for Dansk Pelsdyravlerforening.1999, 37-42.

Radostits, 1994. *Cap. 18 Diseases caused by bacteria III.* In: Radostits, O.M., Blood, D.C., Gay, C.C., Veterinary Medicine. Eight ed. Baillière Tindall, London. p. 703-706.

Rattenborg, E., Clausen, T., Dietz, H.H., 1995. Præliminære resultater af undersøgelse af fedtede hvalpe. (Preliminary results of prospective studies of greasy kits). NJF,Göteborg. *2 pp.*

Schneider, R.R., Hunter, D.B., 1993. A survey of causes of mortality in adult mink, with emphasis on the lactation period. Can. Vet. J., 34, 103 – 108.

Svansson, V., 1991. Studier af en række virusbetingede infektioner hos mink. (Studies of various viral infections in mink). Thesis. The Royal Veterinary and Agricultural University, Copenhagen, Denmark. 150pp.

Svennekjær, N.C., 1954. Minksygdomme og aktuelle problemer i minkavlen (Mink diseases and current problems in mink farming). Dansk Pelsdyravl 12, 384-404.

Torres-Medina, A., 1987. Isolation of atypical rotavirus causing diarrhea in neonatal ferrets. Lab Anim. Sci. 37, 167ff

Trautwein, G.W. & Helmboldt, C.F. 1966. Mastitis in mink due to Staphylococci aureus and *Escherichia coli*. J.Am.Vet.Med.Ass., 924-928.

Uttenthal, Å., Henriksen, P., Østergård, J., Clausen T.N., 1999. Immunglobulinmålinger i mink. (Immunoglobulins in mink) Faglig Årsberetning for Dansk Pelsdyravlerforening.1998, 189-196

Vulfson, L., Pedersen, K., Dietz, H.H., Andersen, T.H., 2000. Infektion med colibacterier hos mink. (Infections with *Escherichia coli* in mink) Faglig Årsberetning for Dansk Pelsdyravlerforening.2000, 179-186.

Wamberg, S., Olesen, C.R:, Hansen, H.O., 1992. Influence of dietary sources of fat on lipid synthesis in mink (*Mustela vison*) mammary tissue. Comp. Biochem. Physiol. 103A, 1, 199-204

II – 14 RP

Oral immunization of fur-bearing animals against salmonellosis

I.a. domski, z.n. beltyukova
Prof. B.M. Zhitkov Russian Research Institute of Game Management and Fur Farming, 79 Engels Street, 610000, Kirov, Russia. E-mail: sable@fur.kirov.ru

Abstract
For oral immunization of fur-bearing animals (Arctic foxes, red foxes, raccoon dogs and nutrias) suppressor streptomycin dependent revertants (*Sal. typhimurium* № 3; *Sal. choleraesuis* № 9; *Sal. dublin* № 6) were used. Lyophilized mixture of above-mentioned vaccine strains was diluted, mixed with feed and given to animals. As a result the innocuity, areactogenicity, antigenic and immune activity of a new method of vaccination of fur-baring animals against salmonellosis were proved. Tests of a new vaccine were carried out under farm conditions. Oral vaccination ensured strong saving of animals from salmonella infection, decreased labour input of farm workers and veterinary specialists. That new method of salmonellosis prophylaxis in fur-bearing animals is recommended for introduction into veterinary practice of fur-bearing animal breeding.

Introduction

Control of infectious pathology was always an important and actual problem. Nowadays the industrial fur-bearing animal breeding requires the veterinary science and practice to use effective means and methods of infectious disease prophylaxis.

Despite the achievements of veterinary science, during recent years in many regions of Russia an abrupt decline of salmonellosis epizootic situation including fur-bearing animals (Koromyslov, 1995) was noted. Cases of salmonellosis in fur-bearing animals were registered earlier (Nordstoga, 1992; Henriksen, 1996).

During recent years attenuated strains of salmonellas (Shuster et al., 1994) were more and more used for the prophylaxis of salmonellosis in agricultural animals and birds because of a low efficiency of inactivated vaccine preparations technological bases of which were developed in the 1960s (Lyubashenko et al.,1964). To improve prophylactic preparations against salmonellosis live vaccine strains were successfully used for fur-bearing animals (Domski et al., 2001, 2001).

Besides, attenuated strains for salmonellosis prophylaxis in animals give an opportunity to use them for oral vaccine prophylaxis. The experience of practical use of such vaccines for agricultural animals is known both in Russia, and abroad. However, there is no information about using oral vaccines in fur-bearing animal breeding.

Materials and Methods

When we carried out that work fur-bearing animals: Arctic foxes, red foxes, raccoon dogs, nutrias and their young at the age of 40-50 days were used. Lyophilizied vaccine for oral immunization was prepared under biofactory conditions. It contained the mixture of attenuated strains of salmonellas of three serotypes: *Sal. typhimurium* № 3; *Sal. choleraesuis* № 9; *Sal. dublin* № 6. All strains were deposited in Russia. The above mentioned types of salmonellas were chosen as virulent vaccine antigens because just those virulent strains are causative agents of salmonellosis in fur-bearing animals in over 80 % of cases.

Vaccine was diluted and added to feed: for carnivora – to meat-fish-grain minced feed, for nutrias – to full-ration granules or stewed grain. Before giving to animals the feed was thoroughly mixed. Vaccine was added in one immunizing dose per one portion of feed and given to animals that were preliminarily put on a 24-hour diet.

Vaccine was singly given to adult animals a month before the rut. In the farms where cases of salmonellosis took place, females were additionally vaccinated at the period of pregnancy, but not later than 14 days before whelping. Young animals were vaccinated twice beginning with an age of 40 days and at an interval of 5-7 days. To study the innocence of vaccine animals were given a 3-fold immunizing dose.

The dynamics of immune response in vaccinated animals was studied with an agglutination test (Antonov, Blinov, 1971) and opsono-phagocytic reaction of neutrophils (Labinskaya, 1978). It is important to note that when carrying out a

phagocytic reaction field virulent strain *Sal. typhimurium* was used as a test object.
Antibody titers were given as geometrical average indices (Lyurski, 1980). Results of an opsono-phagocytic reaction were shown as Striter's number characterizing a phagocytic activity of neutrophils. Those results were processed statistically. Estimation of indices significance was done by Students's criterion (Lakin, 1981). Industrial testing was carried out in 5 fur farms of Russia on 21 thousand individuals of fur-bearing animals of different species.

Results and Discussion

A vaccine-feed mixture was eaten by animals fully and willingly. And changes in animals' behaviour, drop of appetite, refusal of feed, vomiting, alimentary canal upset, signs of depression and disease of animals were not noted. Even when vaccine doses were many times higher than an immunizing one, animals remained clinically healthy and active. Immunization of females did not have a negative impact on the course of pregnancy and normal development of the offspring.
The results of immune reaction studies are given in Tables 1 and 2. To compare the results those tables contain data on parenteral administration of vaccine from attenuated strains.
When studying postvaccine changes in the organisms of animals it was found that different methods of vaccination resulted in the same dynamics of the immune response. Immune indices increased already on the 7 th day after vaccination, their maximal values were noted on the 14 th day. Then their gradual decrease took place. Practically during all the dates there was a certain increase both of specific antibody titers in blood serum of vaccinated animals, and the indices typical of phagocytic activity of leukocytes. Besides, it is

Table 1 Antibody titer indices in agglutination test in fur-bearing animals vaccinated against salmonellosis with different methods

Species of Animals (n=5)	Method of Vaccination	7 days	14 days	21 days	28 days
Fox	Parenteral, double	957.32	1434.0	809.7	271.89
	Oral, double	273.74	1292.6	395.2	180.15
	Control	14.03		13.32	13.27
Arctic fox	Parenteral, double	538.78	1015.9	507.2	113.13
	Oral, double	235.42	978.0	425.4	95.13
	Control	15.8	14.6	15.03	10.0

Table 2 Indices of phagocytic activity of neutrophils in fur-bearing animals vaccinated against salmonellosis with different methods

Groups of Test Animals (n=5)	7 days		14 days		21 days		28 days	
	M± m	P	M± m	P	M± m	P	M± m	P
Fox								
Parenteral	32.6± 2.27	< 0.001	36.2± 2.33	< 0.001	32.6± 0.93	< 0.001	28.5± 0.86	< 0.001
Oral, double	32.0± 3.18	< 0.02	34.0± 2.87	< 0.001	30.2± 52.0	< 0.01	26.2± 1.65	< 0.02
Control	19.4± 1.36		18.5± 1.5		19.4± 1.36		17.7± 1.8	
Arctic fox								
Parenteral	36.0± 6.46	< 0.02	34.2± 2.85	< 0.001	29.5± 2.5	< 0.01	27.5± 1.5	< 0.001
Oral, double	30.0± 1.22	< 0.001	29.2± 1.89	< 0.001	27,7± 0.95	< 0.001	23.5± 2.7	> 0.5
Control	16.5± 1.36		17.5± 0.33		19.5± 1.44		21.0± 0.02	

Table 3.Number of fur animals immunized by oral vaccine against salmonellosis

Fur Farm	Species of Animals	Species of Animals (Heads) Vaccinated between Years				Total Number of Animals (Heads)
		2000	2001	2002	2003	
Fur Farm "Vyatka"	Silver fox	2000	-	-	-	2000
	Red fox	800	-	-	-	800
	Arctic fox	2300	-	8000	-	10300
	Raccoon dog	200	-	400	-	600
Scientific and Industrial Association "Pushnina"	Arctic fox	-	1400	-	1200	2600
	Nutria	71	200	-	-	271
Fur Farm "Pushnoye"	Arctic fox	-	-	3500	-	3500
Fur Farm "Syktyvkarskoye"	Silver fox	-	400	400	400	1200
	Total Number	5371	2000	12300	1600	21271

necessary to point out that in the case of oral vaccination immune indices turned out to be somewhat lower than after intramuscular injection of vaccine.

It was shown that on the basis of investigations carried out earlier (Domski, 2003) titers of specific antibodies in all dates of studying indicated a rather strong immunity to salmonella infection, and it was proved that their level saved the Arctic fox young from disease and death when control infecting with virulent strains of pathogenic organisms took place.

Indices of an opsono-phagocytic reaction showed a strongly pronounced cell immune reaction to the virulent strain and that reaction characterized specificity and trend of immune changes in the organism of fur-bearing animals vaccinated against salmonellosis.

From 2000 till 2004 oral vaccination against salmonellosis was approved under the conditions of Russian fur farms (Table 3).

After carrying out tests under fur farm conditions specialists did not note any after-effects and contraindications to a new method of immunization against salmonellosis. In the fur farms where that method was used the death of animals with the signs of alimentary canal affection decreased 2 times Fecundity, survival and output of whelps per female in different species of fur-bearing animals increased.

Conclusion

The results of studies showed that there were no contraindications for oral immunization of animals against salmonellosis. It was proved the innocence and areactogenicity of a new vaccine.

The above-mentioned data showed that oral immunization of fur-bearing animals against salmonellosis with attenuated strains was an effective and promising way of salmonellosis prophylaxis in industrial fur breeding and gave an opportunity to carry out antiepizootic measures with minimal work input of veterinary specialists.

The results given in that paper, the experience of practical use of an oral method of vaccination of fur-bearing animals against salmonellosis showed that a new method of immunization should be recommended for its introduction into veterinary practice of fur-bearing animal breeding.

Literature

Antonov, V.Ya., Blinova, P.N. 1971. Laboratory investigations in veterinary. Moscow. (in Rus.)

Domski, I.A. 2003. New means and methods of specific prophylaxis of salmonellosis, carnivore distemper and adenoviral infections of fur-bearing animals. Moscow. P. 261 (in Rus.).

Henriksen, P. 1996. Fur animal health – current status. Pages 3-38 *in* Applied Science Reports. 27 Progress in Fur Animal Science. Warsaw.

Koromyslov, G.R. 1985. Scientific investigations on infectious pathology of animals. J. Veterinary. 8:3-7 (in Rus.).

Lakin, G.F. 1981. Biometry. Moscow. P. 105-107 (in Rus.).

Labinskaya, A.S. 1978. Microbiology with the technique of microbiological investigations. Moscow: Medcine. 392 pp. (in Rus.).

Lyubashenko, S.Ya., Petrov A.M. 1962. Diseases of fur-bearing animals. Moscow: Selkhosizdat. P. 17 (in Rus.).

Lyarski, Z. 1980. Diagnostics of Viral Diseases. Pages 148-154, 212-216 (in Rus.).

Manual of Standards for Diagnostic Tests and Vaccines. 2000. Office International des Epizooties (O.I.E.). World Organization for Animal Health. Samonellosis. P. 832-842.

Nordstoga, K.1992. Modern situation of fur animal. Norvegian J. of Agr. Scie. 9:35-38.

Patent of RF № 2166329 of 10.05.2001. Associated Vaccine against Carnivore Distemper and Salmonellosis of Foxes and Arctic Foxes. Domski I.A., Malakhov Yu.A., Ulasov V.I., Panin A.N., Lenev S.V. (in Rus.).

Patent of RF № 2194530 of 20.12.2002. Associated Vaccine for Prophylaxis of Fur Animal Diseases. Domski I.A., Zakharova Ye.D., Ulasov V.I., Malakhov Yu.A., Selovanov A.Ye., Radkov V.N. (in Rus.).

Shuster, B.Yu., Malakhov, Yu.A., Kirillova, V.V., Lebedev, O.A., Lenev, S.V. 1994. Specific prophilaxis of salmonellosis of agricultural animals. J.Veterinary. 2:11 (in Rus.).

SCIENTIFUR
ISSN 0105-2403
Vol. 28, No. 3

Proceedings of the VIII International Scientific Congress in Fur Animal Production

III: Nutrition

Edited by:

Dr. Bert Urlings
Prof. Dr. Berry Spruijt
Dr. Marko Ruis
Ing. Louise Boekhorst

III – 1 RP

A systematic approach to sustainable fur farming with special reference to feed and feeding

Ilpo Pölönen[1], Haiko Koenen[2], Michael Sønderup[3]
[1]Finnish Fur Breeders' Association, P.O.Box 5, FIN-01601 Vantaa, Finland, ilpo.polonen@stkl-fpf.fi
[2]Pecon B.V., Het Frans Brugske 6, 5421 HN Gemert, The Netherlands, Peconb.v.@dapgemert.nl
[3]Danish Agricultural Advisory Service, National Centre Fur animals, Udkaersvej 15 Skejby DK – 8200 Aarhus N, Denmark, mis@landscentret.dk

Introduction

Sustainability and sustainable development are terms that are often used. They were first used by Robert Silvers in 1974 and widely brought into publicity by Bruntlandt Commission in 1987 and later refined and developed more in the United Nations Agenda 21 (Rio de Janeiro 1992). According to Bruntland Commission sustainability means 'meeting the needs of the present without compromising the ability of future generations to meet their own'. More practically sustainability is understood as wise use of natural resources.

Sustainability in fur farming is the key for modern and future fur farming. Sustainability is necessary, not only in reference to new (European) legislation, but also to produce a well-balanced and societal justified fur product. Sustainable fur business means a financially healthy business that is accepted by the society and therefore fulfils the demands of the current and future society.

Sustainable fur production is necessary if we want to maintain fur production in the fur-producing countries. Production must also be both economically and culturally sustainable. If fur breeders cannot accept production conditions and therefore stop producing, it does not matter whether or not production is sustainable in other respects. In that case, production will move to countries where it is sustainable according to other criteria and conditions. Balanced sustainability in all its aspects must be the basis for fur production in the future.

Sustainability in fur animal feeding – fact or false?

Traditionally fur animal farming has been considered sustainable, mainly connected to the type of feed it uses. The feed that mink and foxes consume can not be eaten by other species. Mainly it is made of by-products or even waste that elsewhere would cause significant costs to their producer to dispose it of. Fur industry recycles millions of kilograms of mostly organic phosphorus back into use as fertilizer and thus saves non-renewable phosphorus sources.

What is the cost of recycling, in terms of using non-renewable energy? Would it be more sustainable not to recycle than recycle and what alternatives do we have? All efforts should be evaluated for considering sustainability. Sustainability is a very complex concept where all affects - there is no such concept as absolute sustainability in modern society. Some activities are only more sustainable than others. As mentioned before the use of slaughter by-products contributes to sustainable fur farming. As an example, in 2003 approximately 500 million poultry are slaughtered in the Netherlands, this is equivalent to 690 million kilogram of poultry by-products. To produce a mink skin approximately 35 kg of feed is needed. The Dutch fur animal feed consists, on average during the year, of 65% poultry slaughter by-products, 25% fish by-products and 10% of carbohydrates and premix. Producing 3 million mink skins is then equivalent to the use of 73.5 million kg poultry by-products. Rendering these by-products will cost 5 eurocent per kilogram. Producing mink feed will cost approximately 13 eurocent on energy, logistics and depreciation. Summarizing, for net 8 eurocent per kilogram, a product that is not to be used for human consumption or other animal feed is valorised to a useful feed product. Otherwise approximately 37 million euro has to be spent for rendering these poultry by-products. So mink eating poultry by-products contributes to sustainability of the poultry and the fur business!

There is not necessarily a conflict between an economically and an ecologically sustainable fur production.

General Strategy

How could sustainability be enhanced in fur animal feeding? The answer is 'by strengthening the basic concept, use of by-products, but in a more sustainable way'. Two principles arise above others - saving nutrients, saving energy, enhancing health, well-being and production efficiency. In addition to saving non-renewable mineral sources (e.g. phosphorus) saving nutrients means reduced feeding costs and is therefore also economically sustainable; lower price per kg of feed and lower costs needed to return nutrients (manure) back into circulation. The fact that also saving energy is economically sustainable, gives a good direction and support to the future developments of feeding of fur animals; less use of non-renewable natural energy sources (oil, even electricity) is a core thought of sustainability, together with high efficiency.

Remarkable results in sustainability, even before the idea was introduced, have already been achieved. The first principle, saving nutrients, has long been in the minds of both farmers and researchers. Whereas fur animal feed from this point of view is much more sustainable than it was forty years ago, direction has been right, not much attention was paid to phosphorus, a key element in environmental protection until water protection laws were set in the early 1990's.

Reduced feed consumption per produced area of skin improves the sustainability of fur production. The economy will be improved and the use of energy from production to delivery at the cage of the animal as well as release to the environment in the form of manure and evaporation will be reduced. The reduced impact on the environment will lead to improved acceptance by society.

The large variation in feed requirement at different times of the year is well known. A large spread in the period of birth needs a high degree of individual feeding that puts a very big responsibility to the person in charge with the feeding management. The spread in the peak of birth-date can go up to 10 days. Especially at an advanced stage of lactation the needs of the different farms will differ a lot. Farms with an early birth peak are demanding for a higher energy content opposite to farms that still need the lower energy level in the feed to support the lactation in females with the younger kits and the kits just starting to eat. The broad variation of needs makes it almost necessary to produce two kinds of feed in this period, but the extra labour and energy involved in this goes straight against sustainability. But also other approaches can be useful to provide sustainable solutions. As an example it can be mentioned dividing the animals into weight groups in order to obtain a high whelping result via an easier and more uniform winter condition or in the lactation period the date of birth and the litter size or the daily feed requirement are registered and fed in intervals of , for example, 100 gram. Both methods are used to ensure optimal feeding. The latter feeding method makes it possible to conduct health control and select dams with desirable traits. These initiatives have not resulted in direct selection for improved feed efficiency. Over the years, selection for increased body size and more adapted animals may have increased feed efficiency indirectly. Research results confirm that and show a strong correlation between weight gain and feed efficiency.

When fur farming, in terms of feed manufacturing, grew to industrial size, development work was directed to effectively handling of fish. Big catches required freezing capacity that in economical sense was not in relation to the benefit – a fast and cheap preservation. Fish silage first, and slaughterhouse by-product silage just recently have become economical and more sustainable replacements for freezing, thus saving a lot of energy.

Sustainability, can also be improved by paying specifically attention to the different periods during the production cycle of fur. During late winter and early spring a special attention to animal health and economics is made. Taking care of a perfect animal health and welfare in combination with a good body condition is a base for the start of the mating period and provides the best chance of birth of viable kits. From breeding until nursing precision feeding is necessary to maintain optimal growth with minimum mortality of kits and females. Actually, nowadays a discrepancy between the needs of the females in different lactation stages and the growing kits in reference to the composition of the feed is occurring. Nowadays, the demands for the breeding females are very high, good pelt quality, good size, high fertility and the capacity to nurse many kits. All these mainly commercial demands have to go parallel with an excellent health and a good well-being. If welfare and health are not guaranteed, sustainability is out of the question. Development of the mink feed in combination with good health management is one of the measures taken to fulfil these high demands. In comparison to 20 years ago, a clear adjustment of feed energy levels has occurred. In that time a variation of 1150 to 1400 kcal/kg in feed energy was normal , while at present

a variation through the whole season of 950 to 1700 kcal is the standard in order to provide the mink with optimal feed.
Using the broader variation in feed energy helps to fine-tuning the animal body condition and to supply the animals demands to a larger volume of feed during wintertime in order to secure the welfare. A broader variation in energy can also have a positive effect on the environment, a higher percentage of fat in the feed during summer and autumn provides the farmer a change to perform precision feeding what leads to a lesser amount of manure and decreases the volume and weight leading to a lower logistic pressure to transport the feed from factory to farmer. Still new efforts have to be taken to examine the possibility to reduce the transport of 70% water in the feed.

Energy efficient feeding process
Undoubtedly research towards more sustainable use of nutrients needs to be continued, there is still a lot to be done, but even more remarkable results in sustainability can be achieved by saving energy during the feeding process. Even though dry feed system is not utilizable in large scale 'yet', next step towards higher energy efficiency in feed manufacturing and delivery should be taken without delays. The feed delivery system has not changed almost at all in thirty years. Economical crises have forced it to be changed – number of feed centres has fallen below half what it was. However, many feed centres still work below half capacity and high moisture content fur animal feed is transported to the farms, daily, even in winter. Use of energy, from the receiving raw materials to the distribution of feed on cage wire mesh, is a major challenge in developing sustainability in fur animal feeding. Even though many people think that fur animal feeding deserves a sustainability label already now, many also think that it shouldn't be granted to it for the wasting of energy.

Area ecological model
At present the trend is towards larger scale and efficiency in animal production units, in all animal husbandries. The more animal production concentrates and the larger production units become, the more outspoken becomes also the challenges to manage with environmental and sustainable targets. While use of energy in core production decreases, per unit of product, other costs may increase. One solution to keep up with sustainability is to utilize a model that evaluates nutrient flow on certain geographical area. Production units are placed in such a way that nutrient circulation is optimal and cost effective (to farmer, environment, society, future). The future will show if sustainability will be achieved only in large scale plants – or will new technology provide room also to 'medium size farms'? Much depends on cost structure of fur animal production. Will the policy enhances sustainability in all its aspects (economical, ecological, social and cultural)?
Society's demands for agriculture to reduce its impact on the environment are increasing, especially for productions situated in remoted areas where the environment is often sensitive. Many fur farms are located in such areas. If we are not able to comply with the increased requirements, our production conditions will deteriorate and it will be more difficult to increase existing farm sizes and establish new farms.

Use of by-products stays, use of energy decreases
Recycling is a cornerstone in fur farming and will be an important viewpoint in further development of sustainability in fur animal feeding. Future fur animals will be fed with feedstuffs that are still derived from by-products but that are preserved and stabilized with less use of non-renewable energy sources. Feed stability on one hand but especially storage conditions on farms will be improved which implies that feed is delivered fuel efficiently, year round. Requirements of animals will be better taken care for; bulk feed suitable for all fur animal species and developmental stages won't anymore be the most economical solution. Good storage stability of the feed provides possibilities to manufacture and deliver different feeds e.g. during same week or month. All this imply a higher degree of sustainability in fur animal feeding process.

Animal Health
To control health and production of fur animals at such a specialized farm different actions should occur, including analysis of production disturbances, creating awareness of the farmer, planning, monitoring and corrective measures. These events result in a program, used by the farmer that fits into good mink husbandry practices. To obtain this a systematic approach have to be followed. In the Netherlands the system of hazard analysis critical control points (HACCP) is followed (Urlings, 1999). Weak points in the production cycle of mink are clarified and a general preventive management system is designed and adapted to

individual farms and farmers, nutrition and feeding practices plays a paramount role in these health and welfare management systems.
Health management is realised through vaccination and good farm management. Generally, under farm management is understood good housing, adjust to the specific period and demands, clear breeding systems, but primarily a feeding management that secures health and welbeing.

Present and future possibilities

The conditions for moving fur production into a more sustainable direction are now being created. Through research knowledge is gathered about feed efficiency and its correlation to other characteristics as weight gain, feed consumption and behaviour. It has been shown that feed efficiency measured in the growth period has a relatively high heritability. Moreover, feeding data from practical farms and experiences have been collected. Technical development and the increased focus on the feed consumption have led to new feeding technology being developed. It will make it easy to register, to feed the required amount of feed, to collect and to analyse feeding data. A system called “Individual feeding” is being developed in Denmark. More than 35.000 females and 200.000 mink kits are now on this system on Danish farms.
Individual feeding can be used most of the year. In the winter and spring, a large variation in the feed requirement is seen. The feeding method will contribute to winter condition, flushing and feeding during pregnancy in order to keep the animals in a optmal health shape. It is expected that some farms will attain a better whelping result. During lactation and the early growth period the feeding method will make it easier to satisfy the large variation in feed requirements caused by differences in birth date, litter size, whelp size, sex ratio in the litter, and the ability of the mother to take care of herself and her kits. In the growth period “Individual feeding” will provid a system to feed more precisely and thereby cover the actual feed requirement. A more precise feeding will allow for full expression of the growth potential, thereby increasing the average size of the produced skins, and lead to better selection of new breeding animals, resulting in a higher production level. It is expected that more precise feeding will reduce feed wastage.
Year round individual feeding puts new demands on the farmer. Habits must be changed. In the future, farmers will not merely be satisfied with a high feed intake during the growth period, but will demand knowledge of the resulting production level. Besides the previously mentioned advantages, the farmer will have a better overview of the farm and, thereby, better health control year around. An abnormal feed intake will be easier to register. The system will make it easier to allow different people feed the animals, giving the farmer greater flexibility. The feeding machine and the feed storage conditions must be in good order to ensure delivery of the correct amount of feed as well as palatable feed. Last, but not least, “Individual feeding” requires that the feeding kitchen is able to deliver a homogeneous feed, which is easy to deliver at the cage and which gives a stable feed intake.
The conditions for a more sustainable fur production in all its aspects are present. Many people seem to be interested, but it requires a coordinated effort and a far-reaching strategy in the various fur-producing countries and, preferably, coordination between the countries. We have a common goal. If we work together and make use of the available competences we can achieve much more.

III – 2 RP

Influence of using enzymatic preparations: α-amylase, β-glucanase and xylanase on nutrient digestibility in polar foxes (*Alopex lagopus*)

M. Brzozowski, E. Zakrzewska-Czarnogorska
Department of Animal Breeding, Warsaw Agricultural University - SGGW
02-786 Warsaw, Ciszewskiego 8, Poland e-mail: brzozowskim@delta.sggw.waw.pl

Abstract
The aim of the study was to determine the influence of enzymatic preparations: Bio-Feed Alpha® (active enzymes: α-amylase and β-glucanase), which increase starch hydrolysis and Bio-Feed Wheat® (active enzyme: xylanase), which increases hydrolyzed fractions of fiber from cellular walls, on nutrient digestibility in polar foxes. The enzymatic preparations used in this experiment were added in proportions 200 or 400 mg of each per 1 kg of fresh food for experimental animal groups; 21% of cooked grain was used in the animals' diet. Slightly better results observed in experimental groups were not statistically significant.

Introduction
As typical carnivorous fur animals the polar foxes need meat components in their diet. However, opposite to mink they tolerate higher level of cereal products (20-40% of diet), which are the main source of carbohydrates (Jarosz, 1993). Despite of good digestibility of carbohydrates, foxes do not have any enzymes to hydrolyze fractions of fiber (Slawon, 1997). In carnivorous, as opposed to herbivorous animals, low activity in the gastro–intestinal tract of amylolitic and cellulitic enzymes produced by microorganisms is the main reason why fiber is not degraded (Jarosz, 1996). The fiber level of about 1-2 % of dry matter beneficially influences the apparent digestibility of nutrients (Szymeczko et al., 1996). Fiber doses with more than 3% of dry matter decrease nutrient digestibility (Slawon, 1997). Using enzymatic preparations added to grain components helps degrade fiber fractions from cellular walls and improves digestibility of nutrients inside the cells. They thus help improve animal production and reduce expensive production costs (Krzeminski et al., 1995). Enzymatic preparations are mainly used in poultry and swine feeding with positive effects. Many experiments have been carried out on these species (Flemming et al., 1994; Frankiewicz et al., 1999; Kaoma et al., 1998). In fur animal production, the final product is fur and the quality of it depends on feeding. Using preparations helping nutrient digestion can therefore indirectly influence fur quality of foxes.
The mixture of 3 enzymes: α-amylase, β-glucanase and xylanase were used in this experiment. α-amylase and β-glucanase are active enzymes, which added to barley increase starch hydrolysis. Xylanase, if added to wheat, increases hydrolyzed fractions of fiber from cellular walls. α-amylase and β-glucanase are active enzymes in Bio-Feed Alpha®; xylanaze is active enzyme in Bio-Feed Wheat ®. NovoNordisk produces both enzyme preparations.
The aim of the study was to determine the influence two levels (200 mg and 400 mg per 1 kg of fresh food) of the above mentioned enzymatic preparations on nutrient digestibility indices in polar foxes.

Material and Methods
Experiment was carried out on farm, located 200 km south from Warsaw in Poland, in the period from August 15th to December 10th in two seasons: 2000 and 2001. The study spanned over two periods: growing and maturing of fur coat. In both seasons young polar foxes (male), after weaning, were divided into 2 groups: control (6 cubs) and experimental (6 cubs). Daily diet consist in 79% of meat (poultry offal, contains bone and poultry offal, soft, muscle meat, animal fat) and 21% cooked grain (64 % wheat, 34 % barley). The chemical analyze of used diets and theirs energy value are presented in table 1. Enzymatic preparations Bio-Feed Alpha® and Bio-Feed Wheat® were added to the experimental diet 200 mg each per 1 kg of fresh food (200 g per tone) in 2000 and 400 mg each per 1 kg of fresh food (400 g per tone) in 2001. The fecal for estimating digestibility were collected from each animal by 7 days, in the beginning of September (growing period) and middle of November (furring period), in both seasons. For statistical analyses, a 2-sample t-test was used to test differences between control and experimental groups, separately for each season.

Table 1 Composition and Metabolizable Energy (ME) value (in %) of diets used during both experimental periods

Parameter	Season 2000		Season 2001	
	16.07-15.09 (growing)	16.09-15.12 (fur maturing)	16.07-15.09 (growing)	16.09-15.12 (fur maturing)
Chemical composition, %				
dry matter	30.61	33.98	35.34	38.01
crude protein	10.49	12.97	11.16	12.70
crude fat	6.52	9.73	15.02	13.66
nitrogen-free extract	8.95	6.31	6.21	6.37
crude fibre	0.65	0.50	0.81	0.62
ash	3.98	4.46	2.12	4.65
Energy value, MJ/kg				
Protein	1.97	2.43	2.09	2.38
Fat	2.53	3.78	5.84	5.31
Nitrogen-free extract	1.53	1.08	1.06	1.09
Total	6.03	7.29	8.99	8.78
ME derived from, %				
Protein	32.66	33.33	23.24	27.10
Fat	41.97	51.85	64.97	60.47
Nitrogen-free extract	25.37	14.82	11.79	12.43

Results and Discussion

Chemical composition and energetic value of diets used in the study are presented in table 1. Energy share derived from protein, fat and nitrogen-free extract in diets, was compared with norms for polar foxes presented by Slawon, 1997. Diet used during the growing period in 2000 was well balanced; only the % of ME from protein (32.66%) was below the level of recommendations (35-40% ME). According to experiments carried out by Rimeslatten (after Slawon, 1997), a level of 25% ME from protein is adequate for young polar foxes, which are older than 16 weeks. In younger foxes, age 14-16 weeks, low protein can cause reducing growth, but after this period low level of protein does not influence body weight gain or fur quality. The amount of ME deriving from nitrogen-free extract (14.82%) was lower than in norms (30-40% ME). In 2001 season during growth period the share of ME derived from protein was 23.24%. It was a result of the addition of animal fat, which increased the share of ME from crude fat (64.97%). The share of ME from crude fat was even higher than recommended by the Norwegian norms (up to 60% of ME from fat, Heggset, 2000). During fur maturing period the share of ME derived from fat was lower (60.47%) and share of EM from protein was above minimum level (27.10%). According to Slawon, 1997, by using high fat content and reducing the amount of energy from protein and polysaccharides during the last period before pelting, it is possible to improve the growth of foxes.

Digestibility indices of feeding components were much higher in the experimental group during growing period in both seasons (table 2). Young foxes seem to be very sensitive to the addition of feed supplements during that period. During the fur maturity period no differences were found in digestibility (table 3), although the digestibility indices seemed to be numerically slightly higher in the experimental group.

The addition of enzymatic preparations: α-amylase and β-glucanase (Bio-Feed Alpha®) and xylanase (Bio-Feed Wheat®) at 2 levels: 200 mg/kg and 400 mg/kg fresh food, improved digestibility of diet nutrient during the growing period but did not influence digestibility during the furring period. This is probably due to the low level of fiber (under 1.00% of crude fiber) as result of low share of grain used in diet on the farm (21%).

Table 2 Digestibility of diet nutrients by polar foxes during the growing period (means±sd)

Digestibility %	Season 2000			Season 2001		
	C (N=6)	E (N=6)	P-value	C (N=6)	E (N=6)	P-value
Protein	85.49 ± 5.00	91.10 ± 3.44	0.000***	85.55 ± 4.85	90.70 ± 2.87	0.000***
Fat	97.53 ± 1.01	97.27 ± 0.71	0.000***	97.73 ± 0.95	98.16 ± 0.78	0.038*
Fiber	69.29 ± 9.71	71.06 ±11.21	0.442	67.83 ± 11.32	79.02 ± 5.43	0.000***
Nitrogen-free extract	84.95 ± 4.54	89.68 ± 4.15	0.000***	84.74 ± 5.38	89.780 ± 3.35	0.000***

C – control group
E – experimental group
N – number of animals in group

Table 3 Digestibility of diet nutrients by polar foxes during the furring period (means±sd)

Digestibility %	Season 2000			Season 2001		
	C (N=6)	E (N=6)	P-value	C (N=6)	E (N=6)	P-value
Protein	91.64 ± 3.24	92.22 ± 2.11	0.334	86.55 ± 5.20	87.30 ± 5.56	0.557
Fat	99.10 ± 0.42	99.20 ± 0.34	0.256	98.37 ± 0.82	98.04 ± 0.99	0.128
Fiber	64.05 ± 11.45	60.91 ± 14.56	0.275	63.78 ± 18.50	66.35 ± 9.35	0.459
Nitrogen-free extract	86.41 ± 5.73	86.23 ± 3.84	0.865	78.47 ± 8.23	80.29 ± 9.29	0.382

C – control group
E – experimental group
N – number of animals in group

References

Flemming J.S., Flemming R., Fisher da Silva. A.V., 1994. Use of enzymes in feeding piglets from 28 to 60 days old. Revista do Setor de Ciencias Agrarias, 13: 1-2, 293-296.

Frankiewicz A., Urbaniak M., Potkanski A., Krol M., 1999. Effect of dietary enzymatic preparations on performance of weaners. 26: 3, 175-183.

Heggset O.O. 2000. Standard list of fodder doses for mink and foxes in 2000. Norsk Pelsdyrblad, 2, 46-48.

Jarosz S. J., 1996. Digestion in monogastric fur animals. Scientifur, vol 20, no 3, 283-290.

Jarosz S. J., 1993. Hodowla zwierzat futerkowych PWN, Krakow. (“Fur animals breeding”, in Polish)

Kaoma C., Blaha J., Heger J., Skarkowa L., 1998. Estimation of the optimum level of an enzyme preparation in barley-based diets for broiler chicks. Zivocisna-Vyroba. 43: 3 129-132.

Krzeminkski R., Kulasek G., Leontowicz H., 1995. Enzymy paszowe w zywieniu zwierzat. Magazyn Wet. 4(2). (“Nutrient enzymes in animals” feeding, in Polish)

Slawon J., 1997: Zywienie lisow i norek . PWRiL, Warszawa. (“Foxes and mink feeding”, in Polish)

Szymeczko R., Bieguszewski H., Burlikowska K., 1996. The influence of dietary fiber on nutrient digestibility in polar foxes. Appl. Sci. Reports of Animal Production Review, 28, 67-72.

III – 3 RP

Different ratio between n-6 and n-3 fatty acids in diets for lactating mink (*Mustela vison*) dams – effect on milk and kit tissue fatty acid composition

By Mette Ulf Hansen[1,2], Mikael Lassén[1], Anne Helene Tauson[2], Hilmer Sørensen[3] and Tove Clausen[4]*

*[1] Danish Agricultural Advisory Service, National Centre, Fur Animals, Udkærsvej 15, 8200 Århus N, Denmark. *Corresponding author: muh@landscentret.dk*

[2] The Royal Veterinary and Agricultural University, Department of Animal and Veterinary Basic Sciences, Grønnegaarsdvej 3, 1870, Frederiksberg C, Denmark.

[3] The Royal Veterinary and Agricultural University, Department of Chemistry and Biometry, Thorvaldsensvej 40, 1871 Frederiksberg C, Denmark.

[4] Danish Fur Breeders Research Center, Herningvej 112C, Tvis, Holstebro, Denmark

Abstract

Three groups of female mink were fed experimental diets from mating until weaning of the kits. The experimental diets were supplemented with sunflower oil, rapeseed oil or fish oil as main fat sources in order to achieve different ratios between n-6 and n-3 fatty acids (2.3:1, high (H), 1.5:1, medium (M) and 0.2:1, low (L)). Brain from newborn kits and brain, liver and adipose tissue (obtained from the inguinal region) from 28 days old kits were sampled. Milk samples were taken from females on day 2 and 28 pp. Fatty acid composition of feed, milk and tissues were analysed. Fatty acid composition of milk, liver and adipose tissue reflected dietary treatment, recorded as higher levels of total n-6 fatty acids in the H group and a higher level of n-3 fatty acids in the L group. Fatty acid composition in kit brain tissue was also affected and showed the same pattern as seen in milk and tissues. Docosahexaenoic acid (DHA) and arachidonic acid (AA) were detected in larger amounts in brain tissue than in other tissues. From this study it was concluded that mink milk and body tissues was affected by the maternal dietary fat source and ratio between the fatty acid series. It was also seen that mink brain tissue had high contents of AA and DHA compared to the other organs and milk.

Introduction

Polyunsaturated fatty acids (PUFA) like arachidonic acid (AA, C20: 4 n-6), eicosapentaeonic acid (EPA, C20:5 n-3) and docosahexaenoic acid (DHA, C20:6 n-3) play important roles in the development of the infant brain and retina (Innis, 1991). Linoleic acid (LA, C18: 2 n-6) and AA are members of the n-6 series, because the first double bond is located at the 6th carbon atom from the methyl end. Linolenic acid (ALA, C18: 3 n-3), EPA and DHA belong to the n-3 fatty acid series, because the location of the first double bond is at the 3rd carbon atom from the methyl end. LA and ALA are essential to mammals, because they lack the capability of inserting af double bond beyond the 9th carbon atom from the carboxylic acid end. DHA and AA are formed by elongation and desaturation from ALA acid and LA respectively. The two fatty acids series use the same enzyme systems in desaturation and elongation. This may lead to a competition between the two fatty acid series, and means that high levels of one fatty acid series may block for the modification of the other series and thereby lower the content of one of the fatty acid series in the organism.(Innis, 1991). Fish oil feeding with high levels of n-3 fatty acids to mice was shown to inhibit Δ-6 desaturase activity followed by a decrease in the levels of AA in the tissues (Raz et al., 1997). Low AA content in the foetus may have negative effects on growth and development (Carlson et al., 1991) and this may be a consequence of a low n-6:n-3 ratio.

The long chain PUFA are very important for the development of the foetus, and therefore the foetus has to be supplied from maternal circulation through the placenta. A preferential uptake of long chain PUFA over non-essential fatty acids in the human placenta was reported, and this reflects higher requirements for PUFA than for non-essential fatty acids in the foetus (Dutta-Roy, 2000). Studies with pregnant women have shown positive relations between the maternal plasma phospholipid concentrations of AA, ALA, LA and DHA and the concentrations of these fatty acids in the foetal phospholipids (Elias & Innis, 2001). The selectivity for PUFA may also be regulated at the cellular level

through a selective oxidation of fatty acids and a selective incorporation of the fatty acids into placental phospholipids (Herrera, 2002). There are, however studies that show that the foetus is capable of converting ALA and LA to DHA and AA, but the efficiency of this process is not known (Dutta-Roy, 2000; Green & Yavin, 1993).

It has been documented that deficiency of n-3 polyunsaturated fatty acids in the brain and retina may lead to functional disturbances like impaired learning abilities and visional function (Bourre et al., 1989). Accumulation of DHA in the developing brain and retina is dependent on the dietary intake of n-3 fatty acids in form of the precursor ALA and also its long chain derivatives like EPA and DHA. The supplementation of dietary n-3 fatty acids from fish oils to pregnant and lactating rats have been shown to alter the fatty acid composition of foetal and neonatal rat brain (Yonekubo et al., 1993). Guesnet et al. (1997) have shown that DHA in the brain increases when the content of ALA in the maternal diet increases.

The aim of the present study was to determine the effect of different ratios between the n-6 and n-3 fatty acids in the diet for pregnant and lactating mink on the deposition of fatty acids in foetal and postnatal tissues of the kits It was expected that dietary fat source and ratios between the fatty acids would affect the fatty acid patterns of the milk and body tissues.

Materials and Methods

Seventy-five Scanbrown female mink were divided into 3 groups. The groups comprised of 50% yearlings and 50% adult females. The experiment was carried out from 25. February until weaning of the kits at 8 weeks of age. The animals were housed individually in conventional farm cages with wooden nest boxes. The females were mated from 3. March. They were tried for a second mating 9 days after the first mating. The experimental diets were composed as shown in Table 1. In order to provide the planned ratios between n-6 and n-3 fatty acids at 12.4:1 (high, H), 4,1:1 (medium, M) and 0.25:1 (low, L) the basal diet was supplemented with sunflower oil, rapeseed oil or fish oil.

Table 1 Diet composition (%), and results from chemical analysis of the diets and calculated fatty acid content.

	High (H)	Medium (M)	Low (L)
Fish offal < 3% fat	72.05	72.05	72.05
Wheat, popped 90%<0.5mm	4.20	4.20	4.20
Barley, popped 90%<0.5mm	4.20	4.20	4.20
Haemoglobin meal	3.00	3.00	3.00
Potato protein	2.00	2.00	2.00
Maize gluten meal	3.30	3.30	3.30
Sunflower oil	3.30	3.00	-
Rapeseed oil	2.00	1.00	-
Fish oil	-	1.30	5.30
Water	5.70	5.70	5.70
Vitamin and mineral premix[1]	0.25	0.25	0.25
No. of chemical analyses	5	5	5
Dry matter (DM)	33.0±1.7	33.7±1.8	33.7±1.7
Ash, % of DM	10.5±2.9	8.6±3.3	8.1±2.8
Crude protein, % of DM	49.0±1.6	49.1±1.0	49.3±1.7
Crude fat, % of DM	18.9±2.1	18.6±1.9	18.9±2.3
CHO, % of DM	21.6±2.5	23.7±2.1	23.7±2.4
ME, MJ/kg DM	17.05	17.01	16.90
Vitamin E mg/kg DM	60	60	60

[1] Contains kg $^{-1}$: Vitamins. A: 28,00,500 IE, D_3: 280000 IE E: 24021 IE B_1: 10002 mg, B_2: 4801 mg, B_6: 3201 mg, B_{12}: 16008 mg, calcium-pantothenate: 3207 mg, biotin: 80 mg, folic acid: 241 mg, niacin: 8002 mg, Minerals Fe: 19712 mg, Cu: 1025 mg, Zn: 12561, Mn: 6238 mg.

Milk samples were collected from 5 females in each group on day 2 pp. and on day 28 pp. The same females were used at both samplings. The milk samples were stored at –20°C until analysis. One newborn kit from each of the litters, of females who were used for milk samplings, was sacrificed by decapitation and their brains were removed. On day 28 pp. five kits from each group were killed by an overdose of sodium pentobarbital 20% (Skanderborg Pharmacy) and decapitated. Brain, liver and adipose tissue (obtained from the inguinal region) were removed and stored in plastic bags at -20°C until analysis. Samples of feed, milk, liver, brain and adipose tissue were lyophilised prior to fat extraction. The fat was extracted by using CO_2 supercritical fluid extraction with ethanol as modifier (Speed SE, Applied Separations) except for brain tissue, which was transesterified directly because of very small amounts. Fatty acid profiles of the diets, milk, liver, brain and adipose tissue were determined by gas chromatography (GC-17A Shimadzu, Kyoto, Japan) of the fatty acid methyl esters (FAME). The FAME was prepared by transesterification by sodium hydroxide and boron triflouride (both in methanol) according to a method modified after Morrison and Smith (1964).

Data from fatty acid composition in milk, brain, liver and adipose tissue was analysed in procedure MIXED in SAS (Littell et al, 1996) after the following general model:

$$Y_i = \mu_i + \eta(\text{female}_i) + \kappa_i$$

Where Y_i was the measured response variable fatty acid composition in milk, brain, liver and adipose tissue; at the i'th observation, μ_i was the general mean describes as:

$$\mu_i = \beta(\text{group})$$

and $\eta(\text{female}_i)$ is the random effect of female and κ_i is the residual error.

Results and Discussion

The analyses of fatty acids in the diets showed that the ratios between the n-6 and n-3 fatty acids in the diets were 2.3:1; 1.5:1 and 0.2:1 in the H, M and L groups, respectively, which differs considerably from the planned ratios (Table 2). Thereby the difference in ratios between the H and M diets was rather small.

The dietary fat content is analysed by two different methods. The super critical fluid extraction gives a higher fat content (Table 2), than the traditional soxhlet extraction (Table 1). The explanation to this may be that super critical fluid extraction (with ethanol as modifier) extracts a higher amount of amphiphilic lipids than the traditional method.

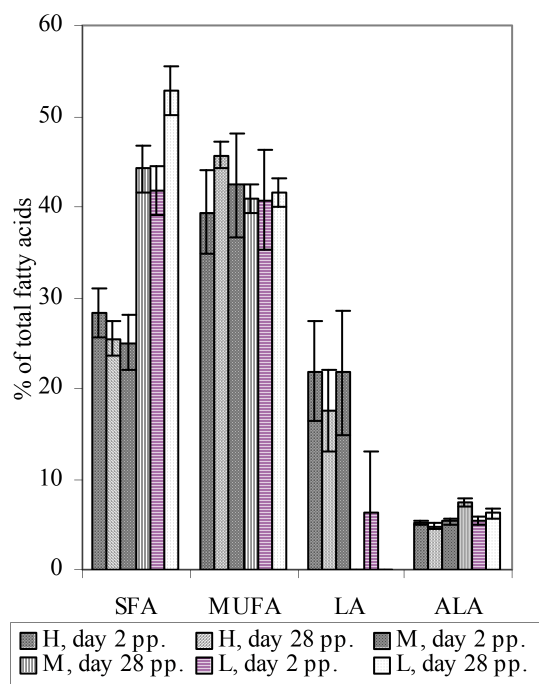
The fatty acid composition of the milk from the L group was characterized by a higher content of SFA, total n-3 PUFA and a lower content of n-6 PUFA compared to the H group (Figure 1). Similar findings from studies with mink were reported by Wamberg et al. (1992) and also in other species dietary fatty acid composition is reflected in the fatty acid pattern of the milk (Yonekubo et al., 1993).

Table 2 Results from analysis of fat content by Super critical fluid extraction (% of DM), fatty acid composition and n-6 and n-3 ratios of the maternal diets (% of total fatty acids). Values are LS-means ± SE (n=3). SFA: saturated fatty acids, MUFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids

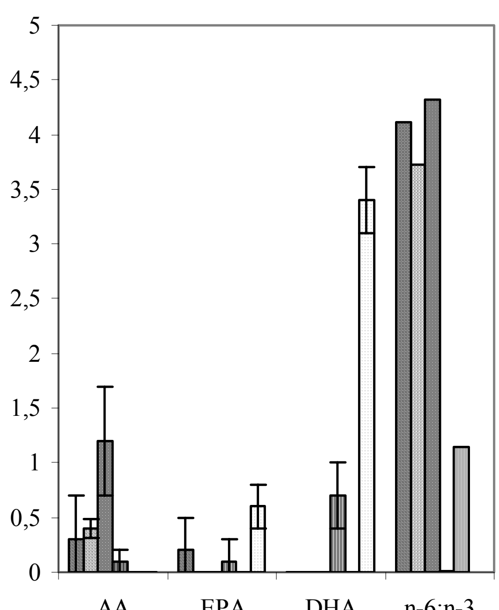
	Dietary treatment		
	High (H)	Medium (M)	Low (L)
Fat % of DM	20.0	19.4	27.9
Total SFA	15.3±0.9	22.3±0.9	34.3±0.9
Total MUFA	53.5±2.5	48.3±2.5	37.6±2.5
Total n-6 PUFA	15.6±3.7	12.9±3.7	3.1±3.7
Total n-3 PUFA	7.0±0.6	8.8±0.6	15.4±0.6
Other	8,6	7,7	9,6
n-6:n-3	2.3:1	1.5:1	0.2:1

Figure 1 A: SFA, MUFA, LA and ALA in mink milk from day 2 and 28 pp. in the H, M and L group (n=5)
1 B: AA, EPA, DHA and n-6:n-3 in mink milk from day 2 and 28 pp. in the H, M and L groups (n=5 per group)

1. A



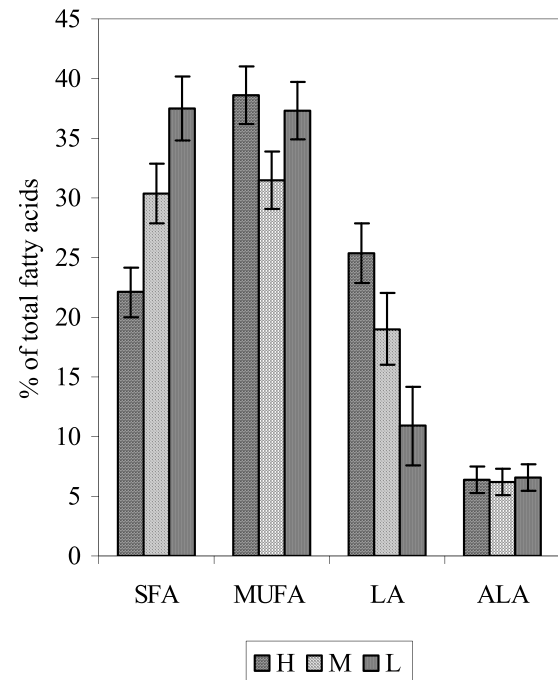
1. B



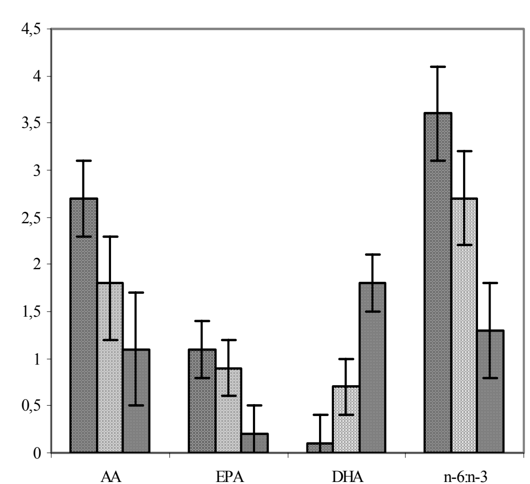
At 28 days of age, the liver fatty acid composition of the kits in the L group was characterized by higher levels of SFA and lower levels of total n-6 PUFA than the other two groups (Figure 2). There were lower levels of both AA and LA in the L group compared to the H and M groups, resembling the fatty acid patterns of the milk. Total n-3 PUFA was not affected by treatment, but the levels of DHA showed significantly higher values in the L group than in the other two groups.

Figure 2 A: SFA, MUFA, LA and ALA in total liver tissue from 28-day-old kits in the H, M and L groups. (n=5 per group).
2 B: AA, EPA, DHA and n-6:n-3 in total liver tissue from 28-day-old kits in the H, M and L groups. (n=5 per group).

2 A



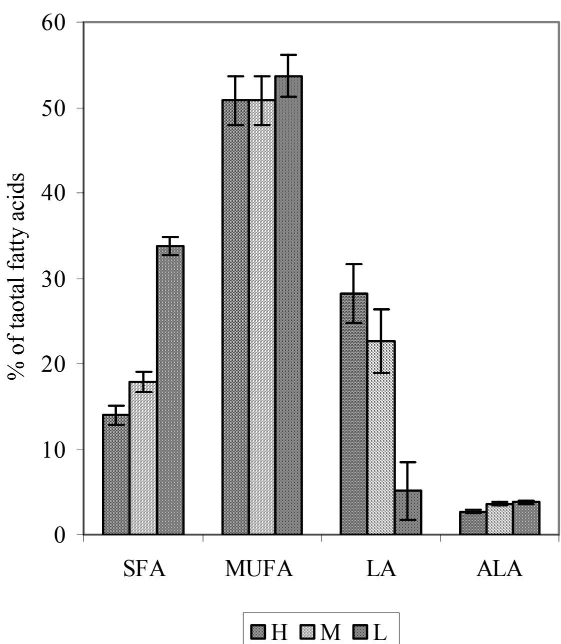
2 B



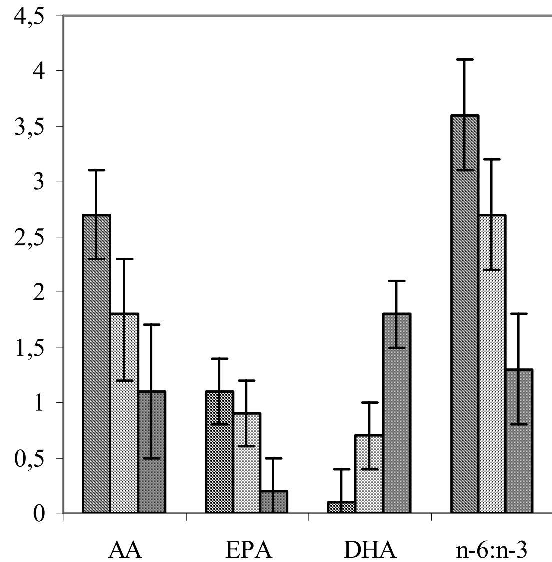
Similar to the milk and liver tissue, the amount of total SFA in the adipose tissue (inguinal region) increased as the ratio between the n-6 and n-3 fatty acids in the diets decreased (Figure 3). The total n-6 fatty acids in the adipose tissue decreased with decreasing dietary ratio, mainly due to a decrease in LA, which was the dominating n-6 PUFA in the adipose tissue. The levels of AA in the adipose tissue were also affected by treatment with higher levels in the H group compared to the M and L groups. The n-3 PUFA were significantly lower in the H group compared to the other groups. Bjergegaard et al. (2003) also reported that incorporation of fatty acids into the adipose tissues of mink kits was highly dependent on the fatty acid composition of the maternal diet.

Figure 3 A: SFA, MUFA, LA and ALA in adipose tissue (inguinal region) from 28-day old kits in the H, M and L groups.
3 B: AA, EPA, DHA and n-6:n-3 in adipose tissue (inguinal region) from 28-day old kits in the H, M and L groups.

3 A



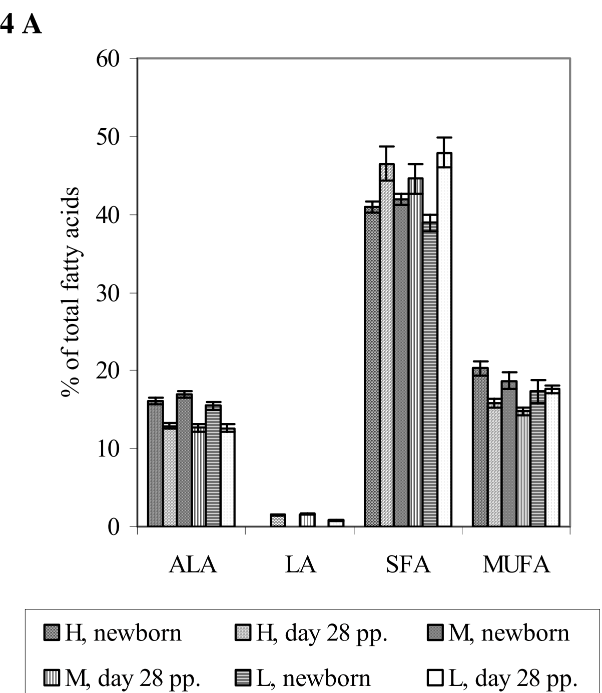
3 B



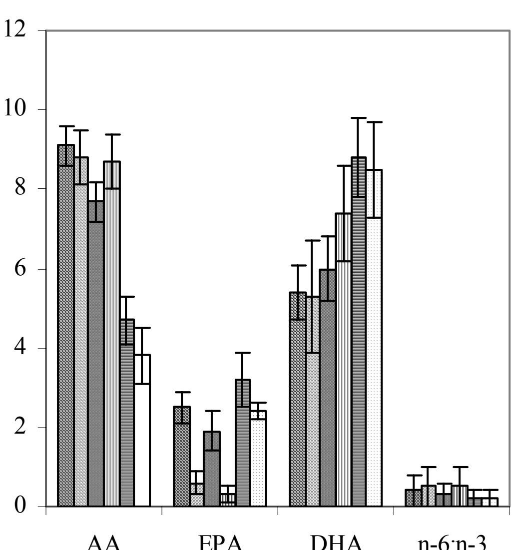
The contents of n-6 and n-3 fatty acids in the brain tissue from the newborn kits were also affected by maternal diet. In the L group the content of total n-6 fatty acids was lower than in the H group, caused by a lower content of AA, because LA was not detectable in the brain tissue from the newborn kits in any of the groups (Figure 4). In brain tissue from the 28 days old kits total n-6 PUFA were significantly lower in the L group compared to the two other groups ($P<0.001$), caused by a lower content of both LA and AA. The content of n-3 fatty acids was, on the contrary, not significantly higher in the L group compared to the H and M groups. Guesnet et al. (1997) found that the most dominating PUFA in the brain tissue in rats were AA and DHA and Hamosh (1997) reported higher levels of AA compared to DHA in the foetal ferret brain. Also in the present study higher levels of AA than of DHA were found in the brain from newborn kits except from the L group where the opposite was found. This indicates that the level of fish oil added in this group was too high to support normal deposition of AA in the brain during pregnancy. The high level of n-3 PUFA may have suppressed the deposition of AA into the tissues and this may have lead to impairment of the brain function. Similar findings were reported by Yonekubo et al. (1993), who found higher levels of DHA in the brain from rats suckling mothers fed a fish-oil diet compared to rats suckling mothers fed a diet without fish oil.

Figure 4 A: ALA, LA, SFA and MUFA in total brain tissue from newborn and 28-day old kits in the H, M and L groups.
4 B: AA, EPA, DHA and n-6:n-3 in total brain tissue from newborn and 28-day old kits in the H, M and L groups.

4 A



4 B



In conclusion, this study showed that the fatty acid composition of maternal diet affects fatty acid composition in milk and kit tissues. Further research is needed to find the importance of the fatty acid deposition in mink fetal tissues.

References

Bjergegaard, C., Clausen, T.N., Lassén, M., Mortensen, K., Sørensen, H., Sørensen, J.C. & Sørensen, S. 2003**:** Growth and development of mink as function of different plant or fish oils included in feed used to mink in the gestation period. NJF-seminar no.354 Lillehammer, Norge 8.-10.October 2003.

Bourre, J., Francois, M., Youyou, A., Dumont, O., Piciotti, M., Pascal, G. & Durand, G. 1989**:** The effects of dietary α-linolenic acid on the composition of nerve membranes, enzymatic activity, amplitude of electrophysiological parameters, resistance to poisons and performance of learning tasks in rats. Journal of Nutrition, 119: 1880-1892.

Carlson, S.E., Cooke, R.J., Rhodes, P.G., Peeples, J.M., Werkman, S.H. & Tolley, E.A. 1991**:** Long-term feeding of formulas high in linolenic acid and marine oil to very low birth weight infants: phospholipid fatty acids. Pediatric Research*,* 30:404-412.

Dutta-Roy, A.K. 2000**:** Transport mechanisms for long-chain polyunsaturated fatty acids in the human placenta. American Journal of Clinical Nutrition, 71, 315s-322s

Elias, S.L. & Innis, S.M. 2001**:** Infant Plasma *Trans,* n-6 and n-3 Fatty Acids and Conjugated Linoleic Acid are related to Maternal Plasma Fatty Acids, Length of gestation, and Birth Weight and Length. American Journal of Clinical Nutrition, 73, 807-814

Green, P. & Yavin, E. 1993**:** Elongation, desaturation, and esterrification of essential fatty acids by fetal rat brain in vivo. Journal of Lipid Research, 34: 2099-2107.

Guesnet, P., Alasnier, C., Alessandri, J. & Durand, G. 1997**:** Modifying the n-3 Fatty Acid Content of the Maternal Diet to Determine the Requirements of the Fetal and Suckling Rat. Lipids 32, 527-534

Herrera, E. 2002**:** Implications of dietary fatty acids during pregnancy on placental, fetal and postnatal development – A review. Placenta 23, Supplement A, s9-s19.

Hamosh, M. 1997**:** Long-chain polyunsaturated fatty acids: who needs them? Biochemical Society Transactions. 26:96-103

Innis, S.M. 1991**:** Essential Fatty Acids in Growth and Development. Progress in Lipid Research 30, 39-103

Littell, R.C., Milliken, G.A., Stroup, W.W. & Wolfinger, R.D. 1996**:** SAS® System for Mixed Models*,* Cary, NC: SAS Institute Inc.

Morrison, W.R. & Smith, L.M. 1964**:** Preparation of fatty acid methyl esters and dimethylacetals from lipids with boron fluoride-methanol. Journal of Lipid Research, 5, 600-608.

Raz,A.;Kamin-Belsky,N., Przedecki,F. & Obukowicz, M.G. 1997**:** Fish oil inhibits delta 6 desturase activity in vivo: Utility in a dietary paradigm to obtain mice depleted of arachidonic acid. Nutritional Biochemistry, 8558-565

Wamberg, S., Olesen, C.R. & Hansen, H.O. 1992**:** Influence of dietary sources of fat on lipid synthesis in mink (*Mustela vision*) mammary tissue. Comparative Biochemistry and Physiology*,* 103A: 199-204.

Yonekubo, A., Honda, S., Okano, M., Takahashi, K. & Yamamoto, Y. 1993**:** Dietary fish oil alters rat milk composition and liver and brain fatty acid composition of fetal and neonatal rats. Journal of Nutrition, 123:1703-1708.

III – 4 RP

Physico-chemical properties of different carbohydrate sources in the gut of mink

H.N. Laerke[1], C. Hejlesen[2] & M.S. Hedemann[1]
[1]Danish Institute of Agricultural Sciences, Department of Animal Nutrition and Physiology, P.O.Box 50, DK-8830 Tjele, Denmark
[2] Danish Furbreeders Research Center, Herningvej 112C, DK-7500 Holstebro, Denmark.
Email: Hellen.laerke@agrsci.dk

Abstract
The carbohydrate sources (CHO) in mink feed may act as controllers of feed intake and satiety. The chemical composition and physico-chemical properties of CHO may change the properties not only of the feed but also the gastrointestinal environment. Studies in other monogastrics have shown that viscosity and water binding capacity (WBC) influence feed intake, rate of passage, enzymatic activity, rate and extent of digestion of nutrients. We studied the in vivo physico-chemical properties of 4 different diets with the CHO comprising either expanded wheat, expanded barley, rolled oats or gelatinized maize starch + sugar beet pulp. The adult male mink were offered 1.26 MJ (300 kcal) metabolisable energy (ME) once daily for 4 days followed by 1.05 MJ (250 kcal) ME for 6-8 days. The rate of feed intake was registered on day 2, 6, and 10. On day 12-14 the mink were euthanized three hours after feeding. The gastrointestinal tract was removed for allometric measurements of the gut, and the contents collected quantitatively for estimation of dry matter content, viscosity, and WBC. The diet containing sugar beet pulp increased WBC of the gut contents, but otherwise the diets showed no or only marginal differences in the gastrointestinal environment. In all groups, there were large variations in the amount and rate of feed consumption with no effects of diet or eating pattern between different days. Higher feed intakes on the day of euthanasia may have been obtained if the mink were fed a meal after overnight fasting although this would have reflected practical farming conditions to a lesser extent.

Introduction

The use of carbohydrate sources in mink feed in practise is often limited to processed cereals like wheat and barley although alternatives have been tested for many years, often with the conclusion that they can be used only in limited amounts (Glem-Hansen & Joergensen, 1978). However, due to environmental and health and welfare considerations renewed interest has been taken in the use of higher proportions of carbohydrate and use of alternative carbohydrate sources in different phases of mink production. It was recently shown that using barley hulls as an energy diluting compound of mink feed increased the time spent on eating and reduced the frequency of stereotypies in mink dams in the winter period without negative effects on production (Hansen et al., 2003). Similar observations have been made previously with pregnant sows fed a diet based on sugar beet pulp (Danielsen & Vestergaard, 2001).

In mink, on the other hand, dietary fibre (DF) is known to have a negative impact on the apparent digestibility of particularly nitrogen (Møller, 1985). Previous studies have also demonstrated a negative impact on mineral balance in mink fed increasing levels of DF from beech, sugar beet pulp and wheat bran due to increased faecal output (Hansen et al., 1985). Broiler chickens are particularly sensitive to variation in DF content and composition, which appears to be associated to a strong influence on the viscosity of the gastrointestinal contents (Svihus & Gullord, 2002). If similar effects are observed in mink gastrointestinal contents, variations in DF content and composition could have negative consequences during the growing period. The current study was performed in order to get preliminary information on the effects of various carbohydrate sources on the physico-chemical properties in the gut of mink.

Materials and methods

Diets

Initially 6 different carbohydrate sources were included in the study; expanded wheat (EW), expanded barley (EB), rolled oats (RO), sugar beet pulp (SBP), pure sugar beet pectin (PP), and pure cellulose (PC). The sources were chosen to cover variation among cereals, SBP as an alternative CHO sources, and PP and PC as pure fibre sources representing contrasting properties of SBP. In the cereal based diets, all CHO came from one cereal

source only. The diets containing high DF ingredients were made to match the dietary fibre (DF) content of a conventional mink feed with the carbohydrates coming from a 50:50 mixture of expanded wheat and barley by supplementation with gelatinised maize starch. Thus the total DF/soluble DF content of the diets were aimed at 4.8/0.9, 7.4/1.8, 4.1/1.8, 6.0/2.5, 6.0/5.8, and 6.0/0.0 % of dry matter (DM), respectively. However, due to low feed intake of the PP and PC diets in the first 4 days, these treatments were excluded in the remainder of the study.
Composition of the 4 experimental diets used in the study is shown in Table 1.

Table 1. Ingredients and energy content of experimental diets.

	EW	EB	RO	SBP
Ingredients before water addition				
Expanded wheat	18.1			
Expanded barley		18.1		
Rolled oats			19.30	
Dried sugar beet pulp				4.40
Maize starch				11.31
Fish meal	2.2	2.2	1.9	4.6
Soy bean oil	3.9	3.9	3.0	3.9
Other ingredients*	75.8	75.8	75.8	75.8
Metabolisable energy, MJ/kg DM§	19.5	18.7	20.9	19.9
Energy distribution	29:50:22	29:50:21	27:55:18	26:55:19

**All diets contained in percentage: Fish offals (<3% fat), 7.7; Industrial fish (5-8% fat), 18.9; Cooked poultry offal, 28.0; Fish silage, 15.0; Haemoglobin, 2.0; Maize gluten, 2.0, 3.9; Lard, 1.9, and Vitamin-mineral premix, 0.3.*
§ Based on chemical analyses and digestibility values presented in Table 4.

Animals and feeding
The animals were kept in cages designed for the measurement of feed intake and faecal output (Jørgensen & Glem-Hansen, 1973) for a total of 12-14 days. Eight adult males per treatment group were offered 1.26 MJ (300 kcal) ME once daily for 4 days followed by 1.05 MJ (250 kcal) ME for 6-8 days. The amount of ingested feed at 4, 8, 10, 12, 14, and 24 hours after the feeding was registered on day 2, 6, and 10. On day 6-10 faeces and feed residues were collected quantitatively and stored at –20ºC until further analysis in order to calculate digestibility of the diets. On day 12-14 the mink were euthanized by an overdose of pentobarbital-Sodium three hours after feeding. The GI tract was removed and separated by ligatures for quantitative collection of contents from the stomach, small intestine, and large intestine. The empty stomach, small intestine, and large intestine were weighed, and the length of the small and large intestine.

Analyses
Viscosity of extracts of the diluted carbohydrate sources (2 g + 8 ml 0.9% NaCl + 0.02% w/w NaN_3 solution) and undiluted diets was determined after incubation at 39ºC for 1 hour followed by centrifugation at 11,400 x g at 4ºC for 20 min. Extract viscosity of digesta was determined by centrifugation immediate after collection. The supernatant was withdrawn and the viscosity (mPa·s) was determined in a Brookfield DV-II cone/plate viscometer (Brookfield Engineering Laboratories, Inc., Stoughton, MA; USA) maintained at 39ºC and a shear rate of 6-60 s^{-1}. Absolute viscosity values are presented at shear rate 45 s^{-1}.
Water binding capacity (WBC) of the undiluted and diluted feeds and carbohydrate sources (2 g + 8 ml 0.9% NaCl + 0.02% w/w NaN_3 solution) was measured as the amount of water withheld per gram of DM after incubation for 20 h at 39ºC and centrifugation at 4,000 x g at 4ºC for 20 min and drying of the sediment. WBC of digesta was determined immediately after collection by centrifugation at 11,400 x g for 20 min at 4 ºC, followed by freeze drying of the sediment.
Swelling capacity of the carbohydrate sources (200 mg) and the diluted diets (1 g) in 0.9% NaCl + 0.02% w/w NaN_3 solution was analysed as the volume (ml) occupied by sample in a 10 ml measuring cylinder after 1 hour at 39°C, and calculated per g of DM.
The feeds and faeces were freeze-dried prior to further analysis. Ash was determined according to AOAC (2000), protein was determined as N x 6.25 by the Dumas method (Hansen, 1989), and HCl- fat according to Stoldt (1952). Without prior extraction

of low-molecular weight sugars, starch was determined essentially as described by Knudsen & Hessov (1995), and DF, including total (T-NSP) and soluble non-starch polysaccharides (S-NSP), according to Knudsen (1997). Duplicate analyses were performed on all samples.

Calculations and statistical analyses
Crude carbohydrates (CHO) were calculated as DM – (crude protein + crude fat + ash). Digestibility of N, fat, and CHO was calculated as the percentage of consumed component not excreted in faeces.
For each separate day percentage of feed eaten was calculated with the feed source as a fixed effect, and time after feeding as fixed repeated measurements using PROC MIXED in SAS software. Response parameters obtained from the day of euthanasia were calculated with the feed source as a fixed effect, and day of euthanasia as a random effect using PROC MIXED with ‘GROUP’ option to account for any differences in variance between dietary treatments. Data calculated from the period of quantitative collection (digestibility) were analysed using the same model but without the random effect. Statistical evaluation of viscosity was performed on logarithmized values, and data presented are geometric means with the corresponding 95 % confidence limits.

Results and Discussion

Physico-chemical properties of carbohydrate sources, diets and gastrointestinal contents
The swelling capacity of the cereals, (wheat, barley, and oats) were similar (7.8-8.1 ml/g DM), whereas the swelling capacity of sugar beet pulp and maize starch were 11.3 and 20.3 ml/g DM, respectively. This was reflected in the diets (Table 2), although absolute differences between diets were smaller. Similarly, expanded wheat and barley had a WBC of 2.9 g water/g DM, whereas the WBC of rolled oats was only 1.7 water/g DM. Sugar beet pulp and maize starch, on the other hand, had higher WBCs (5.0 and 3.7 g/g, respectively). Also these differences were reflected in the diets (Table 2). The extract viscosities of the carbohydrate sources and the diets did not show the same pattern. The batch of expanded barley used in the present study had a very high extract viscosity (111 mPa·s), whereas the viscosity of expanded wheat and sugar beet pulp were very low (2.6 and 1.4 mPa·s, respectively), and autoclaved maize starch had a viscosity of 4.4 mPa·s. Rolled oats had a viscosity of 21.6 mPa·s, but in spite of this, diet RO had an extract viscosity equivalent to diet EW, whereas the viscosity of EB was only 2.5 times the viscosity of diet EW (Table 2).

Table 2. Chemical composition and physico-chemical properties of diets after water addition.

	EW	EB	RO	SBP
Water added (%)	18.7	21.0	13.2	32.1
DM (%)	39.6	37.1	43.1	33.4
Chemical composition, % of DM				
Crude CHO	32.6	32.7	27.3	29.9
Starch	24.1	21.5	23.8	21.3
DF	7.8	12.0	6.3	10.9
T-NSP	4.9	6.7	4.2	6.2
S-NSP	1.5	2.1	2.5	2.7
Klason lignin	2.8	5.3	2.0	4.6
Physico-chemical properties				
Viscosity undiluted, mPa·s	2.9	7.2	2.8	4.1
Swelling diluted, ml/g DM	4.7	5.2	4.9	7.5
WBC undiluted, g water/g DM	2.3	2.6	1.8	3.6
WBC diluted, g water/g DM	1.2	1.2	1.0	1.5

The differences in the physico-chemical properties of the carbohydrates sources and the corresponding diets were only partially reflected in the properties in the gut (Table 3). The WBC of the contents of the stomach and the small and large intestines was significantly higher with the SBP diet than the other diets, whereas the WBC in the stomach of the RO fed mink was numerically, but not significantly, lower. No differences in extract viscosity of the stomach contents were seen, and in the small intestine significantly lower viscosities were measured in mink fed the EB and SBP diets than mink fed the EW diet. These differences could not be explained by differences in the properties of the diet. Some dietary fibre sources may influence the osmotic pressure in the gut by binding water. Negative consequences of this may be increased excretion of Na and K ions (Hansen et al., 1985). Diet-related differences in DM content of stomach contents could have been induced either by increased water intake or differences in secretory

response. However, in the present study we did not observe any significant differences in the DM percentage (Table 3). Due to the limited amount of material collected, this value could not be estimated for the small intestinal contents, but we did not observe any differences in DM content of the faeces collected during 5 days either (Table 4). On the other hand, the generally lower digestibility of DM, organic matter, protein, and particularly ash with the EB and SBP diets indicates that the fibre sources of these diets increase endogenous secretion and reduce the digestibility of other dietary components as previously demonstrated in mink as well as other carnivores (Fekete et al., 2001). In other monogastric animals DF is fermented to varying extent, leading to increased faecal excretion of microbial protein (Eggum, 1992). Although very easily fermentable CHO sources like oligofructose have been shown to increase the density of anaerobic and decrease the number of aerobic bacteria (Williams et al, 1998), fermentation of DF is unlike to add significantly to excreted protein (Børsting et al. 1995) due to a very short rate of food passage, and a generally lower microbial activity bacteria in mink compared to other monogastric animals (Williams et al, 1998).

Feed intake

No differences were seen between the dietary treatments in the rate of feed intake, or eating patterns between the different days (data not shown). However, intra- and inter-individual variations were very big, and on each day 2-5 mink out of 8 per treatment group had ingested less than 95 % of the offered meal within 24 hours. On average, 28, 49, 59, 65, 69 and 83 % of the ration was eaten at 4, 8, 10, 12, 14, 24 h after feeding. Corresponding to this, the mink had consumed 18-32 % of a full ration 3 hours after feeding on the day they were euthanised. The variable feed intake (ranging from 0 to 59 % of the ration offered in the morning) also resulted in vary variable amounts collected from the GIT (2.5-78.7 g). Even from mink who ate nothing or less than 10 g of the diet offered, we observed digesta in the GIT (2.5-38.9 g). Presumable the mink had eaten feed from the previous day just prior to changing feed in the morning. Having fasted the mink overnight would probably have reduced the variability among individuals. However, this strategy would have made results of the study less relevant to practical farming conditions. The relatively low content of DF used in the present experiment might explain why we did not find differences in eating pattern as observed in previous studies with fibre as energy diluting ingredients (Hejlesen & Sandbøl, 2003).

Table 3. Characteristics of gastrointestinal contents.

	EW		EB		RO		SBP		P-value
WBC stomach, g water/g DM	1.57^{a}	*0.12*	1.67^{a}	*0.16*	1.40^{a}	*0.20*	2.39^{b}	*0.24*	0.048
WBC SI, g water/g DM	2.79^{a}	*0.15*	2.76^{a}	*0.28*	3.07^{a}	*0.17*	3.72^{b}	*0.23*	0.007
Viscosity stomach*, mPa·s	1.3	*(0.9-1.8)*	1.2	*(1.0-1.4)*	1.3	*(1.1-1.6)*	1.6	*(0.9-2.9)*	0.303
Viscosity SI*, mPa·s	4.4^{a}	*(3.2-6.0)*	2.5^{b}	*(1.8-3.4)*	3.2^{ab}	*(2.0-5.1)*	2.6^{b}	*(1.7-4.0)*	0.043
Total amount of digesta, g	34.0	*9.4*	47.4	*9.5*	45.9	*8.8*	25.0	*7.6*	0.146
Amount in stomach, g	23.0	*8.0*	35.1	*7.4*	33.7	*7.1*	13.9	*5.1*	0.067
Amount in SI, g	6.9	*1.8*	8.1	*1.8*	8.3	*1.7*	8.0	*2.1*	0.878
Amount in LI, g	4.0	*1.4*	4.2	*1.1*	4.0	*0.8*	3.2	*1.0*	0.861

Values in italics are standard error of means. Values in the same row with different superscripts are significantly different p<0.05. Values in brackets are 95 % confidence intervals obtained from logarithmised values. Values are geometric means. SI, small intestine. LI, large intestine.*

Table 4. Feed intake, faecal output and digestibility of diets.

	EW		EB		RO		SBP		P-value
Intake and output									
Feed intake, g	474[b]	*20*	525[ab]	*34*	432[b]	*32*	620[a]	*47*	0.028
Intake, %	81.3	*3.5*	86.0	*5.6*	79.2	*6.0*	89.0	*6.7*	0.643
Intake DM, g	187.9	*8.1*	194.5	*12.8*	186.1	*14.0*	206.8	*15.5*	0.714
Faeces excreted, g	134.2[bc]	*9.5*	178.3[a]	*12.0*	120.6[c]	*12.5*	162.2[ab]	*12.1*	0.017
Excreted DM, g	37.8[bc]	*2.0*	46.7[a]	*2.9*	33.5[c]	*2.7*	45.7[ab]	*3.3*	0.015
% DM, faeces	28.4	*0.5*	27.1	*2.5*	28.3	*0.9*	29.1	*3.1*	0.956
Digestibility, %									
DM	81.3[b]	*0.6*	77.8[d]	*0.3*	83.4[a]	*0.6*	79.5[c]	*0.3*	
OM	84.2[b]	*0.6*	81.1[d]	*0.3*	85.9[a]	*0.5*	82.8[c]	*0.3*	<0.001
Ash	35.7[a]	*1.9*	28.3[b]	*1.1*	38.9[a]	*2.4*	27.2[b]	*0.7*	0.001
N	83.2[a]	*0.6*	80.4[b]	*0.4*	82.9[a]	*0.6*	80.2[b]	*0.4*	<0.001
Fat	96.2[a]	*0.2*	95.4[b]	*0.1*	95.6[ab]	*0.5*	96.3[a]	*0.2*	0.004
Crude carbohydrates	75.9[b]	*0.9*	70.9[d]	*0.6*	79.4[a]	*0.6*	73.0[c]	*0.6*	<0.001
Starch	98.0[ab]	*0.9*	99.2[b]	*0.0*	99.3[a]	*0.0*	99.4[a]	*0.0*	<0.001

Values in italics are standard error of means. Values in the same row with different superscripts are significantly different p<0.05.

Conclusions

In the present study we observed only minor differences in the physico-chemical properties of the gut contents from mink fed different carbohydrate sources. In practical mink feeds, carbohydrate sources are diluted with other protein- and fat rich sources and water, so differences between cereal sources and different batches of cereal are presumably obscured. However, using higher amounts of fibre rich carbohydrate sources than the SBP diet of the present study is expected to induce differences in physico-chemical properties and consequently effects on eating pattern.

References

Borsting, C.F., Bach Knudsen, K.E., Steenfeldt, S., Mejborn, H. and Eggum, B.O. 1995. The nutritive value of decorticated mill fractions of wheat. 3. Digestibility experiments with boiled and enzyme treated fractions fed to mink. Animal Feed Science and Technology 53**:** 317-336.

Eggum, B.O. 1992. The influence of dietary fibre on protein digestion and utilisation. In: Schweizer, T.F. and Edwards, C.A., eds. *Dietary fibre - a component of food. Nutritional function in health and disease*. Springer-Verlag, London. pp. 154-165.

Danielsen, V. and Vestergaard, E.M. 2001. Dietary fibre for pregnant sows: effect on performance and behaviour. Animal Feed Science and Technology 90:71-80.

Fekete, S., I. Hullar, E. Andrasofszky, Z. Rigo, and T. Berkenyi. 2001. Reduction of the energy density of cat foods by increasing their fibre content with a view to nutrients' digestibility. Journal of Animal Physiology and Animal Nutrition 85:200-204.

Glem-Hansen, N. and G. Joergensen. 1978. Digestibility of feedstuffs determined on mink. Scientifur 2:37-58.

Hansen, B. 1989. Determination of nitrogen as elementary N, an alternative to Kjeldahl. Acta Agriculturae Scandinavica 39:113-118.

Hansen, N. E., S. H. Møller, and H. Mejborn. Fibre containing ingredients in mink feed [Fiberholdige stoffer i pelsdyrfoder]. 85, 13 pp. 1985. Proceedings of NJF Seminar 85, Aalborg, Denmark.

Hansen, S. W., T. N. Clausen, J. Malmkvist, and B. M. Damgaard. Fibres in mink feed in the winter period. 354, 10 pp. 2003. Proceeding of NJF - Seminar 354, Lillehammer, Norway.

Hejlesen, C. and P. Sandbøl. Fibre as a satiety factor in mink feed. 4 pp. 2003. Proceedings of NJF seminar 354, Lillehammer, Norway.

Jørgensen, G. and Glem-Hansen, N. 1973. A cage designed for metablolism- and nitrogen balance trials with mink. Acta Agriculturae Scandinavica 23:3-4.

Knudsen, K. E. B. 1997. Carbohydrate and lignin contents of plant materials used in animal feeding. Animal Feed Science and Technology 67:319-338.

Knudsen, K. E. B. and I. Hessov. 1995. Recovery of inulin from Jerusalem artichoke (*Helianthus tuberosus L.*) in the small intestine of man. British Journal of Nutrition 74:101-113.

Møller, S. H. 1985. The influence of fibre-containing materials in digestibility of nutrients, and excretions of water and minerals in faeces of mink [Fiberholdige stoffers indflydelse på næringsstoffernes fordøjelighed, og minkens udskillelse af vand og salte i gødningen]. Master thesis. The Royal Veterinary and Agricultural University, Copenhagen.

Stoldt, W. 1952. Vorschlag zur Vereinheitlichung der Fettbestimmung in Lebensmitteln. Fette und Seifen/(Fette, Seifen, Anstrichmittel) 54:206-207.

Svihus, B. and M. Gullord. 2002. Effect of chemical content and physical characteristics on nutritional value of wheat, barley and oats for poultry. Animal Feed Science and Technology 102:71-92.

Williams, C., Elnif, J. and Buddington, R.K. 1998. The gastrointestinal bacteria of mink (*Mustela vison L*): influence of age and diet. Acta Veterinaria Scandinavica 39:473-482.

III – 5 RP

Effect of lactic acid bacteria and β-glucanase treatments on the nutritive value of barley for growing blue fox

Jarmo Valaja[1], Ilpo Pölönen[2], Eija Valkonen[1] and Taina Jalava[1]
[1]MTT, Agrifood Research Finland, Animal Nutrition, FIN-31600 Jokioinen, Finland
e-mail:jarmo.valaja@mtt.fi
[2]Finnish Fur Breeders Association, P.O. Box 5, FIN-01601 Vantaa, Finland

Abstract
The effect of lactic acid bacteria or β-glucanase supplements on the chemical composition of mixtures of barley - water or barley –slaughter by-product-water was studied in a laboratory-scale experiment. The effects of cooking, lactic acid bacteria or β-glucanase supplementation on the digestibility of barley diets were evaluated using 20 growing male blue foxes. Barley fed to the foxes was either untreated (control), cooked, lactic acid fermented or supplemented with ß-glucanase enzyme. In the laboratory experiment, the content of total ß-glucans decreased in all barley-water and barley-water-slaughter by-product mixtures during 48 hours. The degradation of total ß-glucans was greatest for the barley-water mixture supplemented with ß-glucanase. In the digestibility experiment, total tract digestibility of carbohydrates and starch were higher for the cooked barley diet (carbohydrates: 63.7% and starch: 72.4%) than for the untreated (carbohydrates: 33.1% and starch: 45.7%), fermented (carbohydrates: 33.7% and starch: 45.0%) or ß-glucanase supplemented barley diets (carbohydrates: 34.0% and starch 43.8%) ($p<0.05$). No differences in the digestibility of carbohydrates or starch were observed between the untreated, fermented or enzyme supplemented barley diets.

Introduction
Barley is the main feed crop in Finland. Unfortunately it has some anti-nutritional properties containing substantial amounts of soluble fibre, mixed-liked β-glucans, which are indigestible for non-ruminants. β-glucans may cause viscous digesta, reduced diet digestibility and cause sticky droppings especially for poultry. Anti-nutritional effects of barley can be reduced by β-glucanase enzyme or treatments such as lactic acid fermentation (Skrede et al. 2001, 2003). Feed enzymes have improved digestibility of diets containing cereals both in mink and dogs (Børsting et al. 1995, Twomey et al. 2003). In blue fox nutrition there is limited experience with β-glucanase enzyme supplementation.
In previous investigations with growing blue foxes, digestibility of carbohydrates in raw barley has been 5-10% lower than that in cooked or heat-treated barley (Kiiskinen et. al. 1988). However, in blue fox growth experiments, high amounts of raw barley have been used successfully (Valaja et al. 2003).
The purpose of this investigation was to evaluate the effects of different chemical and physical treatments on the nutritive value of barley for the growing blue fox. In a laboratory study, the effects of lactic acid fermentation or ß-glucanase supplementation on the chemical composition of barley were studied. In a digestibility study, the effects of cooking, lactic acid fermentation or ß-glucanase supplementation on the digestibility of barley diets by growing blue foxes were studied.

Material and Methods
Laboratory experiment
The effect of lactic acid bacteria or β-glucanase supplementation on the chemical composition of barley-water (1:1.33) and barley-slaughter by-product-water (1:1.66:1.33) mixtures was studied in a laboratory-scale experiment. Barley was ground before mixing using a 2.0 mm sieve. The slaughter by-product was formic acid preserved. The barley-water and barley-slaughter by-product-water mixtures were made as such or supplemented with lactic acid bacteria (Valio AIV Biostart, Valio Ltd; 2 x 10^6 cfu/g mixture) or β-glucanase enzyme (Avizyme 1110, Danisco Nutrition Ltd; 0.45 ml/kg mixture). The mixtures were stored in a 0.5 l plastic bucket with a lid for 24 or 48 hours at room temperature. Three replicates per treatment and time point were used in the study. The mixtures were analysed for β-glucans.

Digestibility experiment
A digestibility experiment was conducted with 20 growing male blue foxes (*Alopex lagopus*) (3.5-4.5 month of age). The animals were housed

individually in digestibility cages throughout the trial. They were allotted according to their initial weight to five blocks of four animals each. The experimental design was incomplete latin-square with four diets and three periods. Each 10-day period contained 7 days of adjustment and 3 days of total collection of faeces. Weighing of the animals was performed at the beginning and the termination of the experiment, as well as at the start and end of each collection period.

The four experimental diets contained either untreated barley (control), or cooked, lactic acid fermented or ß-glucanase enzyme supplemented barley as a source of cereal. The cooked barley was mixed with water (30% barley and 70% water) and cooked for 20 min. Before fermentation the barley was mixed with water (46% barley and 64% water). Lactic acid bacteria product (Valio AIV Biostart,) was mixed with water (1.677 g/2.5 l) and added to the barley-water mixture (555 ml/kg). Fermentation lasted for 24 hours at room temperature. ß-glucanase enzyme (Avizyme 1110) was added to the mixed feed (0.45 ml/kg feed) and the feed was stored overnight in a refrigerator. The diets consisted of the experimental barley (about 50% of diet dry matter), preserved slaughter by-products, fish meal, rape seed oil, methionine and vitamins and minerals (Table 1). The experimental feeds were made all at once and stored frozen pending consumption. Animals were fed once daily at 0900 according to diet dry matter content. Average daily allowance was increased from 772 to 859 g/day during the experiment.

The feeds and faecal samples were analysed for proximal composition, starch, neutral detergent fibre (NDF) and gross energy. Digestibility coefficients were calculated by total collection of faeces. Experimental data were subjected to analysis of variance using GLM procedure of SAS using the following statistical model: $y_{ijkl} = \mu + b_i + b(a)_{ij} + p_k + d_l + (b \times d)_{il} + e_{ijkl}$, where y_{ijkl} is the dependent variable, μ the overall mean, b_i the square effect, $b(a)_{ij}$ the effect of the animal within the square, p_k the effect of period, d_l the effect of the diet, and e_{ijkl} normally distributed random variable.

Results and Discussion

The laboratory experiment revealed that content of total ß-glucans in the barley decreased linearly during the 48-hour incubation (Figure 1) As expected the degradation of the total ß-glucans was the greatest in the barley-water mixture supplemented with the ß-glucanase enzyme, where the amount of the total ß-glucans after 48 hours

Table 1. Dietary ingredients (%) and chemical composition of the diets (g/kg DM).

Treatment	Control dried barley	Cooked barley	Lactic acid fermented barley	ß-glucanase supplemented barley
Ingredient, %				
Acid-treated slaughter by-product	32.00	32.00	32.00	32.00
Barley	21.36	64.48	45.40	21.36
Fish meal	1.00	1.00	1.00	1.00
Vitamins and minerals	0.43	0.43	0.43	0.43
Rape seed oil	2.00	2.00	2.00	2.00
Methionine	0.10	0.10	0.10	0.10
Water	43.12	0.00	19.08	43.12
Composition				
ME, MJ/kg DM	13.95	13.95	13.95	13.95
Dry matter, g/kg	392.6	306.7	380.9	398.2
Crude protein	232.2	240.1	241.8	234.9
Ether extract	177.4	179.4	184.6	175.8
Crude carbohydrates	530.6	517.4	508.5	527.9
Starch	348.0	123.4	332.5	328.6
Total ß-glucans	18.9	21.4	12.7	16.1
Lactic acid	1.73	1.83	11.58	1.61

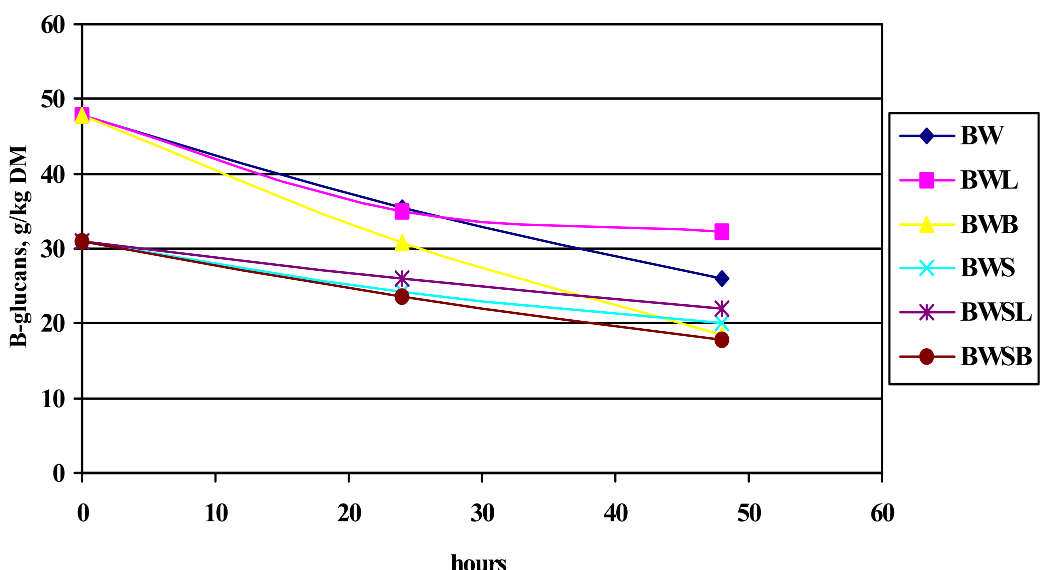
decreased 55% from that in the beginning. The average decrease in the other treatments was between 30-45% after 48 hours. This may be explained by activation of intrinsic ß-glucanase enzymes and lactic acid bacteria by the moisture content to break down soluble fibre components also in barley-water and barley-water-slaugher house by-product mixtures. In earlier studies, fermentation of barley and wheat has been shown to reduce the content of all ß-glucan fractions, whereas lactic acid bacteria preferred the degradation of soluble fraction (Skrede et al. 2001, 2003).

Contrary to what was expected based on the laboratory experiment, the content of the total ß-glucans in barley diets decreased only slightly with the ß-glucanase supplementation (Table 1). Lactic acid fermentation produced lactic acid very efficiently in the diet with fermented barley. Cooking of the barley seemed to degrade starch to sugar components, which resulted in a much lower content of starch in the diet with cooked barley.

All the foxes ate their diets willingly and no refusals were observed. Large differences between the barley treatments were found in the digestibility of carbohydrates (Table 2). The digestibility of starch and crude carbohydrates were clearly the highest in the cooked barley diet ($p<0.05$). Difference in the digestibility of starch varied from 26.7 to 28.8 %-units between the cooked barley and the other treatments. Kiiskinen et al. (1988) also obtained similar differences in the digestibility between cooked and untreated barley diets. There may be several reasons for the low carbohydrate digestibility in the untreated barley diets. Rapid transit time of feed though intestine and insufficient amylase activity may limit digestion of starch. Low starch digestibility in raw barley may also be due to the inability to amylase to penetrate to the starch granules when they are not disrupted.

The digestibility of carbohydrates in the untreated, fermented and ß-glucanase supplemented barley diets were similar. In contrast to our results, Skrede et al. (2001) observed that fermentation of barley clearly improved the digestibility of starch and total carbohydrates in mink compared to untreated barley diets. There are no results so far on the effects of fibre degrading enzymes on the digestibility of barley in blue fox. However, in mink, enzyme treatment has been shown to increase carbohydrate digestibility in whole wheat over that in raw or even boiled wheat (Børsting et al. 1995). In dogs, an enzyme product containing β-glucanase improved digestibility of energy and ether extract of diets containing 51% of barley (Twomey et al.

Figure 1. Effect of different physical and chemical treatments on content of total ß-glucans in barley



(Treatments: BW: Barley+water, BWL: Barley+water+lactic acid bacteria, BWB: Barley+water+β-glucanase enzyme, BWS: Barley+water+slaughter by-product, BWSL: Barley+water+slaughter by-product+lactic acid bacteria, BWSB: Barley+water+slaughter by-product+β-glucanase enzyme).

2003). In our experiment, the conditions for the β-glucanase enzyme were not optimal, since the feeds were frozen. According to Børsting et al. (1995) exogenous enzymes are likely inactive in frozen or cold conditions.

The metabolizable energy (ME) content of the barley diets calculated based on the digestibility results revealed great differences between the cooked barley and the other treatments (Table 2). These differences were likely caused by incomplete carbohydrate digestibility. Practical growth experiments have shown that blue foxes fed diets with high content of raw barley grew as well as those fed diets with low content of barley (Valaja et al. 2003). In our current experiment, the daily gain of the foxes fed the cooked barley was clearly higher than that of foxes fed the other diets ($p<0.05$). In practice, the foxes may increase their feed intake when fed diets with a low dietary ME-value.

In conclusion, cooking clearly improved digestibility of carbohydrates in barley whereas lactic acid fermentation or β-glucanase supplementation had no effect on carbohydrate digestibility.

Table 2. Effect of different treatments on the digestibility of barley diets (%) (LS-means presented).

Treatment	Control, dried barley	Cooked barley	Lactic acid fermented barley	ß-glucanase supplemented barley	SEM
Organic matter	52.4^{b}	70.9^{a}	55.2^{b}	53.7^{b}	0.74
Crude protein	69.1^{b}	73.8^{a}	73.9^{a}	71.0^{b}	0.69
Ether extract	88.2	88.1	90.0	89.8	0.59
Crude carbohydrates	33.1^{b}	63.7^{a}	33.7^{b}	34.0^{b}	1.12
Starch	45.7^{b}	72.4^{a}	45.0^{b}	43.8^{b}	1.20
NDF	20.0^{a}	22.7^{a}	13.2^{b}	21.8^{a}	1.57
ME, MJ/kg DM	12.1	15.1	12.8	12.4	
Daily weight gain, g	36.2^{b}	69.7^{a}	38.7^{b}	45.7^{b}	3.66

[a,b,c] Means within the same row with the same superscript do not differ significantly ($p>0.05$).
SEM=standard error of the means.NDF=neutral detergent fibre.

References

Børsting, C.F., Bach Knudsen, K.E., Steenfeld, S., Mejborn, H. & Eggum, B.O. 1995. The nutritive value of decorticated mill fractions of wheat. 3. Digestibility experiments with boiled and enzyme treated fractions fed to mink. Anim. Feed Sci. Technol. 53: 317-336.

Kiiskinen, T., Mäkelä, J. & Rouvinen, K. 1988. Eri viljalajien sulavuus minkeillä ja siniketuilla (Digestibility of different grains in mink and blue fox). Maatalouden tutkimuskeskus, Tiedote 5/88, p. 14-23.

Skrede, G., Sahlstrøm, S., Skrede, A., Holck, A. & Slinde, E. 2001. Effect of lactic acid fermentation of wheat and barley whole meal flour on carbohydrate composition and digestibility in mink. Anim. Feed Sci. Technol. 90: 199-212.

Skrede, G., Herstadt, O., Sahlstrøm, S., Holck, A., Slinde, E. & Skrede, A. 2003. Effects of lactic acid fermentation on wheat and barley carbohydrate composition and production performance in the chicken. Anim. Feed Sci. Technol. 105:135-148.

Twomey, L.N., Pluske, J.R., Rowe, J.B., Choct, M., Brown, W., Mc Connell, M.F. & Pethick, D.W. 2003. The effects of increasing levels of soluble non-starch polysaccharides and inclusion of feed enzymes in dog diets on faecal quality and digestibility. Anim. Feed. Sci. Technol. 108: 71-82.

Valaja, J., Pölönen, I., Nenonen, N. & Jalava, T. 2003. Diets containing high amounts of barley for growing blue foxes. NJF-seminar 354, 8-10 October, 2003, Lillehammer, Norway. 5 p.

III – 6 RP

Ideal Protein for Mink (Mustela vison) in the Growing and Furring Periods.

P. Sandbol, T.N. Clausen & C. Hejlesen
Danish Fur Breeders Research Center, Holstebro, Denmark. e-mail: ps.pfc@cfc.dk

Abstract

The investigation aimed at establishing the optimal protein requirement for mink in the growing and the furring periods. Based on the present amino acid norm for mink and cat and the amino acid composition of whole mink, an ideal protein was constructed.

Two trials of each 5 groups of 120 males were carried out. Each male was housed with a female. A basal feed containing 32:55:13 % of the metabolizable energy (ME) from protein, fat and carbohydrate, was composed. Animals were weighed monthly and dead animals authopsied. At pelting livers were sampled and the pelts were graded.

In trial 1 the diets contained 32, 28, 24, 20 and 16 % of ME from protein. The lower levels of protein were achieved by substituting the protein fraction with pregelatinized maize starch and fat. The diets had identical amino acid profiles and almost identical energy contents. Based on earlier results from the furring season, Methionine Hydroxy Analog (MHA) was used instead of dl-methionine. The diets were fed from July to pelting. The longest skins ($p<0.0001$) were found in the groups with 24, 28 and 32 % of ME from protein. And the best pelt quality in the groups with 28 and 32 % of ME from protein.

In trial 2 all groups received a diet containing 22 % of ME from protein from July to September. From September to pelting the diets contained 30, 26, 22, 18 and 14 % of ME from protein. There was no difference in skin length and only the group with 14 % of ME from protein had significantly lower pelt quality ($p<0.0006$) as compared to the other groups.

It is concluded, that with the used amino acid profile (including MHA) the optimal skin length is achieved already at 24 % of ME from protein and the optimal pelt quality from 28 % of ME from protein during the whole period. For the furring period, it seems that the requirement may be as low as 18% of ME from protein. In a parallel trial MHA showed inferior results in the growing period and we can not exclude that the effects found, are mere methionine responses.

Introduction

Animals utilize protein for such different purposes, as maintenance, growth (including feathers and fur), reproduction and milk production. The ideal amino acid profiles of the protein used for these different purposes, are distinctly different (i.e. Fuller et al., 1989 and Rodehutscord et al., 1997). Consequently, the ideal amino acid profile changes during an animals growth period, as the maintenance requirement increases and that for growth decreases. For fur bearing animals this is further influenced by the onset of winter fur growth.

It has been known for many years, that the amino acid profile of milk or whole body, is a good basis for the determination of the ideal amino acid profile, i.e. Kim & Lall (2000) and Rollin et al., (2003).

A comparison, of the amino acid profile, of the present norm for mink (N) (Børsting & Clausen, 1996 and Børsting, 1998) in the growing-furring period, with that of mink body and pelt (M) (Glem-Hansen & Hansen, 1981 and Chavez, 1980) reveals a

Table 1. Amino acid profile (relative to lysine) of mink norm (N), mink body+pelt (M), catnorm (C) and "Ideal Protein" (IP)

	N [1)]	M [2)]	C [3)]	IP
Arg	115	119	125	115
Cys	22	67	44	39
Met	59	32	50	47
M+C	81	99	94	86
His	56	38	38	40
Ile	96	54	63	65
Leu	185	122	150	150
Val	130	78	75	80
Lys	100	100	100	100
Phe	107	62	50	65
Tyr	67	56	56	65
Thr	70	74	88	70
Trp	22	22	19	22

[1)] Børsting & Clausen, (1996) and Børsting, (1998); [2)] modified from Glem-Hansen & Hansen, (1981) and Chavez, (1980); [3)] NRC, (1986).

considerable difference for certain amino acids, especially for those where only a so called maximum norm has been established.
Mink are often compared with cats and the amino acid profile of the cat norm (C) (NRC, 1996) is relatively close to either that of the mink norm or that of the mink body plus fur.
An ideal protein (IP) was constructed, based on the present mink norm, the amino acid profile of mink body plus fur and the present cat norm (Table 1).
Two trials were carried out to establish the optimal protein requirement of this ideal protein in respectively the growing and the furring periods.
Generally the trials, which formed the basis of the present amino acid norms for mink, suggested that a level of protein lower than 25% of ME was not supporting the need of the mink in the growing period. For most of these low-protein-diets it was characteristic, that they had a sub-content of one or more amino acids compared to the norms later specified. Rasmussen & Børsting (2000) proved that a low content of protein (21% of ME from protein) influenced the pelt quality negatively, when this was given as feed from the age of 22 weeks, and furthermore there was a tendency of declining growth the sooner it was given in the growing period. By way of comparison a feed with 34% of ME from protein was used. This had an excess of 10% of the sulphur-containing amino acids and from 20 to 90% of the rest of the essential amino acids compared to the norms at present. The diet with 21% of ME from protein was only containing 70% of the sulphur-containing amino acids and from 75 to 106% of the rest of the essential amino acids compared to the present norms.
Apart from the sulphur-containing amino acids, the present norms are the same for both the growing and the furring periods.
Estimating the need of protein on the present norm for essential amino acids plus a corresponding amount of protein from non-essential amino acids, you will find, that the protein need of the mink ought to be in the order of 20% of ME from protein. The corresponding need for cats is estimated to be in the order of 16% of ME from protein (Andersen et al., 1980) considering dry feed with 19.7 MJ ME/kg. Smalley et al. (1985) concluded that the need was higher in terms of protein/kg feed. They used a feed with 21.0 MJ ME/kg and correcting for this and the digestibility of the feed, that has been used, their results will end up to be between 13.0 and 18.6 and their recommendations between 13.8 and 17.0% of ME from protein with 19.7 MJ ME/kg Corresponding to this, Burger et al. (1984) found that the need of maintenance for cats was covered at 12% of ME from protein.
Based on the norms at present for mink during the growing period, the composition of the mink body and the present amino acids profile for cats, trials are carried out in order to shed light on the optimum protein-level in the growing and furring periods respectively.

Materials and Methods

A trial with 120 Scanbrown males in each group was carried out. Each male was housed with a female kit in a two row open house with 6 cages per section and free access to drinking water and nesting box. The animals were pelted from the 10th to the 12th of November.

Feed

The feed was compounded with a so-called ideal protein based on the present mink norm and the amino acid profile of mink body plus fur and the present cat norm.
The decline of the protein was achieved with a proportional decline of all protein-sources and they were regulated with maize starch. The decline of specific protein-sources which led to removal of fat, was adjusted by adding fat with a corresponding fatty acid profile. This should eliminate a possible influence from the composition of the fat. The feeds were analysed at the analytical laboratory of Danish Fur Animal Feed according to official EU methods and the ME content calculated using the following values: 18.8 kJ/g of digestible Crude Protein, 39.8 kJ/ g of digestible Crude Fat and 17.6 kJ/ g of digestibleCarbohydrate (Calculated by difference).

Table 2. From the middle of September until furring we use levels from 30 to 14% from protein and from 15 to 31% from carbohydrate respectively.

<table>
<tr><th colspan="2">Energy Distribution</th></tr>
<tr><th>9th July
- 15th of September</th><th>15th of September
- Pelting</th></tr>
<tr><td colspan="2">32:55:13</td></tr>
<tr><td colspan="2">28:55:17</td></tr>
<tr><td colspan="2">24:55:21</td></tr>
<tr><td colspan="2">20:55:25</td></tr>
<tr><td colspan="2">16:55:29</td></tr>
<tr><td rowspan="5">22:55:23</td><td>30:55:15</td></tr>
<tr><td>26:55:19</td></tr>
<tr><td>22:55:23</td></tr>
<tr><td>18:55:27</td></tr>
<tr><td>14:55:31</td></tr>
</table>

During the period from early July until the middle of September we used feed, where the ME from protein made up between 32 and 16% with a decline of 4 % units for each group. ME from carbohydrate correspondingly increased from 13 to 29% and ME from fat was kept constant on 55%.
From the middle of September until pelting we used levels from 30 to 14% from protein and from 15 to 31% from carbohydrate respectively. The trial setup is shown in table 2, the feed composition in tables 3 & 4 and the calculated amino acid content in table 5. At 20% of ME from protein the norm for essential amino acids was fulfilled compared to the “Ideal Protein”, but compared to the present norms, some of the amino acids were not fulfilled until 24 or 28% of ME from protein. Essential amino acid : total amino acid ratio was 0.54.

Statistics

Statistical analyses of data were carried out by means of the SAS data recording and processing system (SAS, 1988). Differences in body weight gain and skin length were compared using a one-way analysis of variance (GLM procedure). Pelt quality, pelt colour and clarity were tested using a non-parametric test (GENMOD). Skin length was used as a covariate when testing pelt quality. Silkiness and wool quality were tested using the Chi-Square test.

Table 3. Composition for the growing-furring-period-trial with protein requirement for mink kits, in % of diet.

ME from protein, %	32	28	24	20	16
Fish Offal	5.7	5.1	4.5	3.8	3.1
Poultry Offal	27.2	24.2	21.2	18.1	14.8
Slaughter House Offal	35	31.2	27.3	23.3	19.1
Popped Barley	4.0	3.5	3.1	2.6	2.2
Popped Wheat	4.0	3.5	3.1	2.6	2.2
Maize Starch		3.4	7.0	10.7	14.7
Feather Meal	1.8	1.6	1.4	1.2	1.0
Blood Meal	0.5	0.4	0.4	0.3	0.3
Peas	6.0	5.3	4.7	4.0	3.3
Potato Protein	3.0	2.7	2.3	2.0	1.6
Maize Gluten	3.9	3.48	3.05	2.60	2.1
Protao	0.9	0.76	0.66	0.57	0.5
Soya Bean Oil	5.0	5.63	6.36	7.12	7.9
Lard	2.5	2.8	3.2	3.6	4.0
MHA, Methionine Value *	0.54	0.48	0.42	0.36	0.29
Tryptophan	0.062	0.055	0.048	0.041	0.034
Lysine	0.039	0.035	0.030	0.026	0.021
Threonine	0.081	0.072	0.063	0.054	0.044
Vitamins/Minerals	0.25	0.25	0.25	0.25	0.25
Water		5.5	11.0	16.8	22.8
Analytical Composition Energy: MJ / kg MJ / kg DM Dry Matter, % Energy Distribution Ash, %	 9.16 19.9 46 32:54:14 1.7	 9.42 20.2 46.6 29:53:18 1.7	 9.84 20.6 47.8 25:53:22 1.5	 10.64 20.9 50.9 20:52:28 1.4	 11.21 21.4 52.4 18:52:30 1.2

The feed with 32% ME from protein was used as basic blend. From this one the other blends are mixed by adding fat and maize starch.
***NB!** We used Methionine-Hydroxy-Analog instead of methionine, to avoid a toxic effect when adding too much methionine, % addition: 0.304 – 0.271 – 0.237 – 0.202 – 0.165 – 0.21.*

Table 4. Composition for the furring-period-trial with protein requirement for mink kits, in % of diet.

ME from protein, %	30	26	22	18	14
Fish Offal	5.4	4.8	4.1	3.5	2.8
Poultry Offal	25.7	22.8	19.7	16.5	13.12
Slaughter House Offal	33.0	29.3	25.3	21.2	16.9
Popped Barley	3.7	3.3	2.9	2.4	1.9
Popped Wheat	3.7	3.3	2.9	2.4	1.9
Maize Starch	1.7	5.2	8.9	12.7	16.7
Feather Meal	1.7	1.5	1.3	1.1	0.8
Blood Meal	0.4	0.4	0.3	0.3	0.2
Peas	5.7	5.0	4.3	3.6	2.9
Potato Protein	2.8	2.5	2.2	1.8	1.45
Maize Gluten	3.7	3.3	2.8	2.4	1.9
Protao	0.8	0.7	0.6	0.5	0.4
Soya Bean Oil	5.3	6.0	6.7	7.5	8.3
Lard	2.6	3.0	3.4	3.8	4.2
MHA, Methionine Value *	0.51	0.45	0.39	0.33	0.26
Tryptophan	0.058	0.052	0.045	0.038	0.030
Lysine	0.037	0.033	0.028	0.024	0.019
Threonine	0.076	0.068	0.059	0.049	0.039
Vitamins/Minerals	0.25	0.25	0.25	0.25	0.25
Water	2.8	8.2	13.9	19.8	26.0
Analytical Composition					
Energy:					
MJ / kg	9.10	9.49	10.13	10.36	10.64
MJ / kg DM	19.6	20.4	21.0	21.3	21.7
Dry Matter, %	46.5	46.5	48.3	48.7	49.2
Energy Distribution	29:52:19	26:53:21	22:54:24	19:53:28	16:53:31
Ash, %	1.7	1.5	1.4	1.3	1.4

The feed with 32% ME from protein was used as basic blend. From this one the other blends are mixed by adding fat and maize starch.
***NB!** We used Methionine-Hydroxy-Analog instead of methionine, to avoid a toxic effect when adding too much methionine, % addition: 0.304 – 0.271 – 0.237 – 0.202 – 0.165 – 0.21.*

Table 5. Estimated content of amino acids in gram/MJ during the growing period, compared to an Ideal Protein (IP) and the present norm.

						22						
ME from protein, %	32	28	24	20	16	→ 30	→ 26	→ 22	→ 18	→ 14	IP	Present Norm
Met incl, MHA*	1.09	0.92	0.80	0.67	0.54	1.00	0.88	0.75	0.59	0.46	0.67	0.67
Met	0.59	0.52	0.44	0.37	0.30	0.54	0.49	0.41	0.33	0.26	0.67	0.67
Cys	0.40	0.35	0.30	0.25	0.20	0.38	0.33	0.28	0.23	0.18	0.25	0.25
Lys	1.80	1.59	1.34	1.13	0.92	1.72	1.46	1.26	1.00	0.80	1.13	1.13
Thr	1.26	1.13	0.96	0.80	0.63	1.17	1.05	0.88	0.71	0.54	0.80	0.80
Trp	0.40	0.35	0.30	0.25	0.20	0.37	0.32	0.27	0.23	0.18	0.25	0.25
His	0.75	0.63	0.54	0.46	0.37	0.67	0.59	0.50	0.41	0.33	0.46	0.63
Phe	1.55	1.38	1.17	0.96	0.80	1.46	1.26	1.09	0.88	0.67	0.92	1.21
Tyr	1.21	1.05	0.92	0.75	0.59	1.13	0.96	0.84	0.67	0.54	0.75	0.75
Leu	2.93	2.55	2.22	1.84	1.46	2.76	2.39	2.01	1.63	1.30	1.67	2.09
Ile	1.34	1.17	1.00	0.84	0.67	1.26	1.09	0.92	0.75	0.59	0.71	1.09
Val	1.80	1.55	1.34	1.13	0.88	1.67	1.46	1.21	1.00	0.80	0.92	1.46
Arg	2.09	1.80	1.55	1.30	1.05	1.97	1.67	1.42	1.17	0.92	1.30	1.30
Gly	2.01	1.76	1.51	1.26	1.00	1.88	1.63	1.38	1.13	0.88		
Ala	1.93	1.67	1.42	1.21	0.96	1.80	1.55	1.30	1.09	0.84		
Ser	1.59	1.38	1.17	1.00	0.80	1.46	1.30	1.09	0.88	0.71		
Asp	2.43	2.09	1.80	1.51	1.21	2.26	1.97	1.67	1.34	1.05		
Glu	4.35	3.81	3.26	2.72	2.18	4.06	3.52	2.97	2.43	1.88		
Pro	2.01	1.76	1.51	1.26	1.00	1.88	1.63	1.38	1.13	0.88		

* *Estimated content of Met after addition of Methionine-Hydroxy-Analog.*

Results

Due to the way of diluting the protein content of the diets, amino acids were only analyzed in three of the diets. The calculated contents of digestible amino acids/MJ based on these results, showed that the results are within the acceptable deviations due to analytical error. The results are not shown.

The was no statistical significant difference in the initial weights between any of the groups. The calculated weight gains are shown in table 6.

Discussion

In the first series, where the ME from protein ranged from 32 to 18% during the whole period from early July until pelting, we noticed a better growth and longer skins when the ME from protein was in the area of 25-32%. A smaller content of protein caused less growth and shorter skins (Tables 6 & 7). The quality of the skin culminated at 29-32% of ME from protein, and there was most silky and good pelts at 25-32% of ME from protein (Tables 7 & 8). The norm of essential amino acids compared to the “ideal protein” was fulfilled at 20% of ME from protein (Table 5). Compared to the present norm this was not the case for all amino acids before we reached 25 or 29% of ME from protein (His - Ile - Leu .- Val - Phe - Tyr). The reason for these low results of production at 20% of ME from protein, might be the fact that either the proposed IP is too low for some of the amino acids or the total content of protein was too low. Anyhow at 18% of ME from protein the content of amino acids was below the present norm of the first 6 essential amino acids (Arg - Cys - Met - Lys - Thr - Trp).

When ME from protein was 18-20%, there was an increased number of dead kits authopsied with fatty liver (Table 9). However, at the time of pelting there was no difference in neither the Hepato-Somatic-Index, nor the fat content of the livers in the different groups (Table 10).

In the second test series with 22% of ME from protein in all groups until the 15th of September and then variations from 16-29% until pelting, there was no difference in their growth during the whole period, but every single group had less growth from early July till 13th of August compared to the corresponding groups in the first test series (Table 6). We have not been able to find a reason why, but there must be a systematical difference between the 2 series. The length of the furs in the second trial series were not different and only the group, that had

Table 6. Weight gains for male kits, fed with different levels of ME from protein respectively during the growing-furring period or the furring period

ME from Protein, %	Weight gain, g														
	9th July - 13th Aug.			13th Aug. - 4th Sept.			4th Sept. - 25th Sept.			25th Sept. - Pelting			9th July - Pelting		
32	820 (107)	BC	BC	407 (99)	AB	A	436 (118)	A	A	254 (138)	AB		1920 (299)	A	A
29	858 (135)	A	A	416 (136)	A	A	428 (117)	AB	A	222 (180)	AB		1927 (340)	A	A
25	853 (146)	AB	AB	369 (118)	C	B	409 (134)	ABC	A	266 (178)	A		1913 (360)	A	A
20	805 (158)	CD	C	358 (121)	C	B	435 (131)	A	A	209 (179)	BC		1799 (361)	B	B
18	679 (159)	F	D	310 (152)	D	C	345 (169)	D	B	234 (235)	AB		1581 (400)	E	C
22 → 29 from Sep.	778 (147)	DE		383 (131)	BC		378 (138)	CD	BC	255 (151)	AB	A	1803 (309)	BC	
22 → 26 from Sep.	769 (144)	E		384 (109)	BC		420 (124)	AB	A	218 (172)	BC	AB	1800 (310)	BCD	
22 → 22 from Sep.	779 (153)	DE		389 (119)	ABC		401 (122)	BC	AB	218 (183)	B	A	1799 (363)	BCD	
22 → 19 from Sep.	773 (122)	DE		376 (115)	BC		427 (132)	AB	A	137 (251)	D	C	1714 (324)	D	
22 → 16 from Sep.	792 (135)	CDE		405 (113)	AB		352 (157)	D	C	167 (180)	CD	BC	1725 (302)	CD	
P-values: All 32 – 16 22 → 29 - 16		*** 	 *** NS		*** 	 *** NS		*** 	 *** ***		*** 	 NS ***		*** 	 *** NS

The groups in the second series started on a diet with 22 % of ME from protein and the feed was changed over a period from 12th – 15th of September.
*The bracketed figures are the standard deviations. Different letters in a column indicate a statistically significant difference. NS indicates no difference. ***: P<0.001.*

Skin length and fur quality are shown in table 7, and number of silky furs plus wool quality in table 8.

Table 7. Length, quality, colour and clarity of the pelts from the male kits, fed with different levels of ME from protein respectively during the growing-furring period or the furring period

ME from Protein, %	Number of furs	Length, cm			Quality, 1-12 *			Colour, 1-5 #			Clarity 1-5 ¤		
32	107	86.1 (4.5)	AB	AB	7.5 (2.3)	A	A	3.2 (1.0)			2.9 (1.0)	CDE	
29	109	86.5 (4.2)	A	AB	7.2 (2.2)	AB	AB	2.9 (1.1)			2.8 (1.0)	DEF	
25	105	86.6 (4.8)	A	A	6.9 (2.5)	BC	B	3.1 (1.0)			2.9 (1.0)	CDEF	
20	105	85.3 (4.2)	BC	B	6.4 (2.1)	DE	C	2.9 (1.0)			2.8 (1.1)	EF	
18	104	82.6 (5.8)	D	C	5.6 (2.3)	F	D	3.2 (1.0)			2.6 (1.0)	F	
22 → 29 from Sep.	103	84.9 (4.2)	BC		7.1 (1.9)	BC	A	3.0 (0.9)			3.1 (0.9)	BC	B
22 → 26 from Sep.	103	85.0 (4.3)	BC		6.6 (2.3)	CD	A	2.9 (1.0)			3.0 (1.0)	BCDE	B
22 → 22 from Sep.	104	85.5 (5.0)	BC		6.9 (2.2)	BCD	A	2.9 (1.0)			3.2 (1.0)	AB	AB
22 → 19 from Sep.	103	84.7 (4.3)	C		6.7 (1.9)	CD	A	2.9 (1.0)			3.1 (0.9)	BC	B
22 → 16 from Sep.	96	84.6 (4.4)	C		5.9 (2.2)	EF	B	3.0 (0.9)			3.4 (0.9)	A	A
P-values: All			***			***			NS			***	
32 – 16				***			***			NS			NS
22 → 29 - 16				NS			***			NS			**

**12 is best, # 5 is darkest, ¤ 5 is most reddish*
*The bracketed figures are the standard deviations. Different letters in a column indicate a statistically significant dif - ference. NS indicates no difference. ***: P<0.001 and **: P<0.05.*

16% of OE from protein had a poorer fur quality than the other groups (Table 7). There were very few silky and only few furs with good wool in all the groups compared to the first test series (Table 8). This indicates that 22% of ME from protein in the first part of the growing period is too low to achieve a fine quality of the fur, probably because the initial development of the winter fur is beginning as early as July-August. Furthermore it seems that variations in content of protein during the last part of the period does not have the same importance as it had in the first test series, as only the quality of the fur at 16% of ME from protein differed from the others.
When the share of ME from protein was 16%, there was an increased number of dead kits authopsied with fatty liver (Table 9). However, at the time of pelting there was no difference in neither the Hepato-Somatic-Index, nor the fat content of the livers in this group as compared to all of the groups in series one (Table 10).

Table 8. The frequency of silky, flat and full furs of the male kits.

ME from Protein, %	Furs, n	Silky, %	Wool, %		
			Flat	Normal	Good
32	107	11.2	10.2	74.8	15.0
29	109	13.8	14.7	74.3	11.0
25	105	11.4	8.6	77.1	14.3
20	105	8.6	12.4	81.9	5.7
18	104	2.9	16.4	77.9	5.8
22 → 29 from Sep.	103	6.8	13.6	76.7	9.7
22 → 26 from Sep.	103	1.9	7.8	83.5	8.7
22 → 22 from Sep.	104	4.8	17.3	77.9	4.8
22 → 19 from Sep.	103	3.9	12.6	84.5	2.9
22 → 16 from Sep.	96	4.2	16.7	81.3	2.1
P-values:					
All		**	**		
32 – 16		NS	**		
22 → 29 - 16		NS	NS		

*The bracketed figures are the standard deviations. Different letters in a column indicate a statistically significant difference. NS indicates no difference. **: P≤0.05*

Mortalities are reported in table 9.

Table 9. The frequency of dead kits (males and females).

ME from Protein, %	Dead kits, n	
	Total	With fatty liver
32	4	0
29	0	0
25	3	0
20	5	2
18	4	3
22 → 29 from Sep.	1	0
22 → 26 from Sep.	0	0
22 → 22 from Sep.	1	0
22 → 19 from Sep.	0	0
22 → 16 from Sep.	9	4

The results from the liver analysis are shown in table 10.

Table 10.Hepato-Somatic-Index (liver weight in % of body weight), liver dry matter (DM) and calculated liver fat (%) (liver fat, % = 1,15 * liver DM – 24,9)*.

ME from protein, %	HSI, %	Liver DM, %	Liver fat, %
32	2,33 (0,20)	31,9 (2,8)	11,8 (3,2)
28	2,39 (0,32)	30,6 (1,4)	10,4 (1,6)
24	2,23 (0,18)	31,2 (2,0)	11,1 (2,3)
20	2,28 (0,23)	31,6 (2,0)	11,5 (2,3)
16	2,34 (0,27)	30,5 (3,0)	10,2 (3,4)
22 → 14 from Sep.	2,45 (0,28)	31,9 (2,6)	11,9 (3,0)
P-value	NS	NS	NS

** Calculation according to Clausen & Sandbol (2004). The bracketed figures are the standard deviations. NS indicates no difference.*

The results above indicate, that the optimal protein content with an "ideal protein" is in the range of 25-32% of ME. As a consequence, at the lower end of this range, one or more of the amino acids with so-called max. norms might have been first limiting.
Based on an earlier trial in the furring period (Sandbol et al., 2003a) we used MHA instead of dl-Methionine, to avoid any risks associated with excess methionine. However results from Sandbol et al. (2003a & 2003b) indicate, that the requirement for methionine *per se*, may be 20% below the recent norm, and our conclusion (Sandbol et al., 2003a) as to the utilisation of MHA may be wrong. A resent trial (Sandbol et al., 2004) indicates that mink are not or only partially able to utilize MHA in the growing period. If this holds, the response found in the first series may be a response to methionine *per se*.

Conclusion

Considering that Methionine-Hydroxy-Analog is utilized by the mink, the optimal protein content for growth with an "ideal protein" is about 26% of Metabolizable Energy from protein and one or more of the Amino Acid's with present max. norm have been first limiting. Considering that Methionine-Hydroxy-Analog is not, or only partially utilized by the mink, the result found in the first series may be a response to methionine *per se*. Further investigations are required to clarify the mink's ability to

utilize Methionine-Hydroxy-Analog. The requirement for ideal protein in the furring period seems to be well below the present levels used in practical feeding.

References

Anderson, P.A., Baker, D.H., Sherry, P.A. & Corbin, J.E., (1980). Nitrogen requirement of the kitten. Am. J. Vet. Res., 41, 1646-1649.

Burger, I.H., Blaza, S.E., Kendall, P.T. & Smith, P.M., (1984). The protein requirement of adult cats for maintenance. Feline practice, 14, 8-14.

Børsting, C.F., (1998). Ernæringens indflydelse på skindkvaliteten. Intern Rapport Nr. 111, Danmarks JorbrugsForskning. 55-62.

Børsting, C.F. & Clausen, T.N., (1996). Requirements of essential amino acids for mink in the growing-furring period. Proc. VI Int. Sci. Cong. Fur Anim. Prod. (Eds. A. Frindt and M. Brzozowski), Warsaw, Poland. Anim. Prod. Rev., Appl. Sci. Rep., 28, 15-24.

Chavez, E.R, (1980). Amino-acids profile of the plasma, pelt and hair of the adult mink. 2nd Int. Sci. Cong. Fur Anim. Prod., Copenhagen.

Clausen, T., & Sandbol, P., (2004). Correlation between liver fat and dry matter. In “Proc. VIIIth Int. Sci. Cong. Fru Anim. Prod., 2004, Holland. (Poster Presentation).

Fuller, M.F., McWilliam, R., Wang, T.C. & Giles, L.R., (1989). The optimum dietary amino acid pattern for growing pigs. 2. Requirements for maintenance and for tissue protein accretion. Br. J. Nutr., 62, 255-267.

Glem-Hansen, N. & N. Enggaard Hansen, (1981). Amino acid deposition in mink during the growth period. Acta Agric. Scand., 31, 410-414.

Kim, J.D. & S.P. Lall, (2000). Amino acid composition of whole body tissue of Atlantic halibut *(Hippoglossus hippoglossus)*, yellowtail flounder *(Pleuronectes ferruginea)* and Japanese flounder *(*Paralichthus *olivaceus)*. Aquaculture, 187, 367-373.

NRC, National Research Council (1986). Nutrient requirements of cats. National Academy Press, Washingthon, DC.

Rasmussen, P.V. & Børsting, C.F., (2000). Effects of variations in dietary protein levels on hair growth and pelt quality in mink (*Mustela vison*). Can. J. Anim. Sci., 80, 633-642.

Rodehutscord, M., Becker, A., Pack, M. & E. Pfeffer, (1997). Response of rainbow trout *(Oncorhynchus mykiss)* to supplements of individual amino acids in a semipurified diet, including an estimate of the maintenance requirement for essential amino acids. J. Nutr., 126, 1166-1175.

Rollin, X., Mambrini, M., Abboudi, T., Larondelle, Y. & Kaushik, S.J., (2003). The optimum dietary indispensable amino acid pattern for growing Atlantic salmon (*Salmo salar* L.) fry. Br. J. Nutr., 90, 865-876.

Sandbol, P. Clausen, T.N., & Hejlesen, C., (2003a). Methionine and methyldonors for mink (*Mustela vison*). NJF-Seminar no. 354, Lillehammer, Norway, October 8th - 10th, 2003, pp. 5. (Poster presentation).

Sandbol, P. Clausen, T.N., & Hejlesen, C., (2003b). Amino acid profiles in the furring period of mink (*Mustela vison*) in the furring period. NJF-Seminar no. 354, Lillehammer, Norway, October 8th - 10th, 2003, pp. 6. (Poster presentation).

Sandbol, P. Clausen, T.N., & Hejlesen, C., (2004). Svovlholdige aminosyrer til mink i vækstperioden. In “Samlet oversigt over fodringsforsøg I vækstperioden 2003”, 28-34. Pelsdyrerhvervets Forsøgs- og ForskningsCenter, (Intern Rapport). Interrim report in Danish.

SAS (1988), SAS Institute inc. SAS/STAT™ Users Guide. Release 6.03, Edition 1988, Cary, North Carolina.

Smalley, K.A., Rogers, Q.R., Morris, J.G. & Eslinger, L.L., (1985). The nitrogen requirement of the weanling kitten. Br. J. Nutr., 53, 501-512.

III – 7 RP

Effect of feeding intensity on body condition and glycemic control in mink *Mustela vison*

K. Rouvinen-Watt[1], J. P. Murphy[1] and C. Chan[2]
[1]Canadian Centre for Fur Animal Research, Nova Scotia Agricultural College, Department of Plant and Animal Sciences, Truro, Nova Scotia,
[2]Atlantic Veterinary College, Department of Biomedical Sciences, Charlottetown, Prince Edward Island, Canada
Email: krouvinen@nsac.ns.ca

Abstract
Thirty kits from five litters, one male and one female from each, were allocated to three feeding regimes: 80, 100 or 120% of the recommended dietary allowance (RDA) of ME. The mink were weighed (BW), scored for body condition (BCS, scale 1-5, 3=ideal), and sampled for blood and urine monthly from mid-August (start) to mid-December (end). In December, 6/10 mink in the 100%RDA group scored 3, while in the 80%RDA group 7/10 mink received a BCS 2 (thin), and in the 120%RDA group 8/10 mink had a BCS 4-5 (heavy-obese)($P<0.001$). The final blood glucose levels of all mink in the 120%RDA group were higher (6.59mmol l^{-1}) in comparison to the 80%RDA (5.21mmol l^{-1}) and the 100%RDA groups (4.95mmol l^{-1})(SEM=0.37, P#0.01). The males in the 120%RDA group showed hyperinsulinemia (2.06ng ml^{-1}, SEM=0.164, $P=0.043$) in comparison to the rest of the mink (range 1.17-1.51ng ml^{-1}). No glucosuria was detected. The development of obesity appears to be associated with elevated blood glucose concentrations and hyperinsulinemia in the mink suggesting insulin resistance.

Introduction

In the mink *Mustela vison*, the relationship between obesity and glycemic control has not been characterized. The species shows significant fluctuation in body weight in response to seasonal changes in the nutritional and hormonal status, being the slimmest during summer and the heaviest during winter (Korhonen & Niemelä, 1998, Tauson & Forsberg, 2002). In other carnivore species, such as dogs and cats, obesity has been shown to result in poor glycemic control (Hand et al., 2000). It increases the levels of non-esterified fatty acids in blood circulation, which in turn decrease glucose metabolism in other tissues leading to hyperglycemia (Frayn, 2001). This may eventually result in the development of insulin resistance (Frayn, 2001). According to Plotnick and Greco (1995), there is a positive relationship between obesity and blood glucose levels in dogs. Hyperglycemia and the presence of glucose in the urine indicates that blood glucose concentration has exceeded the renal absorptive threshold and that there is potential for development of more serious disorders (Hoenig & Ferguson, 1989). It has recently been proposed that in the mink the history of obesity and the associated development of insulin resistance may be a key predisposing factor to the later development of nursing sickness (Rouvinen-Watt, 2003), the etiology of which is strongly linked to poor glycemic control. The objectives of this research were to study the effect of feeding intensity on body weight, body condition, feed intake, and selected blood and urine parameters in juvenile male and female mink, and to evaluate the impact of obesity on glycemic control.

Materials and Methods

Experimental design, weighing and body condition scoring

Thirty (30) mink kits from five litters, 3 male and 3 female kits in each, were selected for this research. One male and one female mink from each litter were allocated to three different feeding intensity regimes (Table 1) and fed at 80%, 100% or 120% of the recommended dietary allowance (RDA) of metabolizable energy (ME) (NRC, 1982). The experiment lasted from mid-August until mid-December. The early growth diet fed during August contained 17.4 MJ of ME with an energy distribution

between fat, protein and carbohydrates (CP:CF:CHO) 34:44:22. From September until December a diet containing 19.2 MJ of ME (CP:CF:CHO 32:53:15) was fed. The diet included 40% of cod and haddock racks, 25% of chicken necks and backs, 5% liver, 5-10% eggs, 1-2% corn gluten meal, 10-14% extruded wheat, 1-4% fat supplement (herring oil, canola oil, poultry grease) and a vitamin-mineral premix. The mink were housed individually in a conventional two-row shed with a nest-box attached to a rearing cage and adequate bedding material provided. Freshly mixed feed was provided daily according to the designated feeding intensity regime. The amount of feed dry matter consumed daily per mink was measured once a month over a three-day period. The mink were weighed to the nearest 0.1g at the beginning of the project and every four weeks thereafter using a catching cage. The mink were manually caught and restrained in compliance with standard animal management practices (CCAC, 1993). Prior to weighing the mink were scored for body condition using a five-point scale, where Body Condition Score (BCS) 1 = very thin, 2 = thin, 3 = ideal, 4 = heavy, and 5 = obese (Rouvinen-Watt & Armstrong, 2002).

Blood and urine collection and analysis

Urine samples were collected at the beginning of the experiment, in September and in December by placing the mink into a metabolism cage until urine was voided. Each sample was analysed using a DiaScreen 1G urine glucose test strip (MEDgenesis). A blood sample was then taken by toenail clipping and analysed using an Accu-Chek Compact blood glucose monitor (Roche Diagnostics). The blood and urine samples were collected post-prandially and were obtained within 1-2 hours. The exact time from feeding was not measured except at the end of the experiment. The blood and urine were collected over a three-day period, with 12 mink per day being used, including an equal number of males and females from each test group. At the end of the experiment in December, the blood glucose was measured post-prandially prior to anaesthesia, from a sample collected from a clipped toenail, as described above. The time from feeding to anaesthesia was on average 102± 26 min. The mink were anaesthetized using 0.17 ml kg^{-1} BW of Rompun® (xylazine 20 mg ml^{-1}) and 0.09 ml kg^{-1} BW of Ketalean® (ketamine hydrochloride 100 mg ml^{-1}). Blood samples were obtained by cardiac puncture for serum clinical-chemistry (7mL Vacutainer® tubes) and haematology (5mL Vacutainer® EDTA tubes). The mink were then euthanized with an intracardiac injection of Euthanyl® (pentobarbitol 240 mg ml^{-1}, 0.44ml kg^{-1} BW) for subsequent organ and tissue sampling. The blood samples were analysed for haematology and clinical chemistry at the Veterinary Services Laboratory, Nova Scotia Department of Agriculture and Fisheries, Truro, NS. The insulin, triacylglycerol and free fatty acid analyses were carried out in the Obesity and Diabetes Lab at the Atlantic Veterinary College, Charlottetown, PEI. The insulin radioimmunoassay utilized rat insulin as standard; serial dilution of mink serum generated a curve parallel to the rat insulin standard curve, indicating similar cross-reactivity with the antiserum. The antisera was guinea-pig anti-insulin, a kind gift of R. A. Pederson, Vancouver, Canada. Triacylglycerol and free fatty acids were determined using commercially available kits (Diagnostic Chemicals, Charlottetown, Canada and Roche, Laval, Canada, respectively).

Statistical analysis

The MIXED procedure in SAS was used to examine the effects of the sex, treatment, and month and their interactions on body weight, energy consumption, and blood and urine parameters of the mink (Littell et al., 1998). In the model, the litter of origin was used as a random statement and the month as a repeated statement. Where significant effects were detected ($P< 0.05$), the lsmeans± SEM values were tested using the PDiff test. The frequency distribution of the body condition scores in the different treatment groups was evaluated using the Fisher’s exact test.

Results and Discussion

Body weight and energy consumption

In August, at the start of the experiment, the body weights of the mink in the different feeding regimes did not differ. The males (1904 g) were on average significantly heavier than the females (1130 g, SEM=73.0, $P< 0.001$). In September, the males in the 80%RDA group differed from the males in the 100%RDA ($P=0.024$) and the 120%RDA ($P= 0.002$) groups. In October, all male feeding regime groups differed from each other (Figure 1) ($P< 0.05$), whereas in November also the female groups showed

significant differences with the exception of the 100%RDA and 120%RDA groups. At the end of the experiment in December, all groups differed from each other in BW except the 100%RDA and 120%RDA female groups (females: 80%RDA 1144g; 100%RDA 1395g; 120%RDA 1502g; males: 80%RDA 1440g; 100%RDA 2377g; 120%RDA 2755g; SEM=104, P#0.05). The daily energy intake of the mink (Figure 2) differed significantly between some of the feeding regimes already in August, measured one week after the start of the experiment. In September, all male feeding regime groups differed in their energy intake (P#0.021), whereas the females did not show significant differences. In October, all male and female feeding regime groups were significantly different from each other in their diet energy consumption (P<0.05). In November, the male 120%RDA group differed from the other two male groups (P<0.05), whereas the females no longer showed differences among the feeding regimes.

Figure 1.
Body weight of juvenile male and female mink in different feeding regime groups based on Recommended Dietary Allowance. The males are presented with closed and the females with open symbols. The symbols used are as follows: circle 80%RDA, triangle 100%RDA, and square 120%RDA. Presented are lsmeans∀SEM. Planned comparisons: August all groups P>0.05; September males: 80%RDA-100%RDA P=0.02 80%RDA-120%RDA P=0.002; October males: all groups P<0.05; November females: 80%RDA-100%RDA P=0.013, 80%RDA-120%RDA P<0.001, males: all groups P#0.004; December females: 80%RDA-100%RDA P=0.052, 80%RDA-120%RDA P=0.006; males: all groups P<0.01.

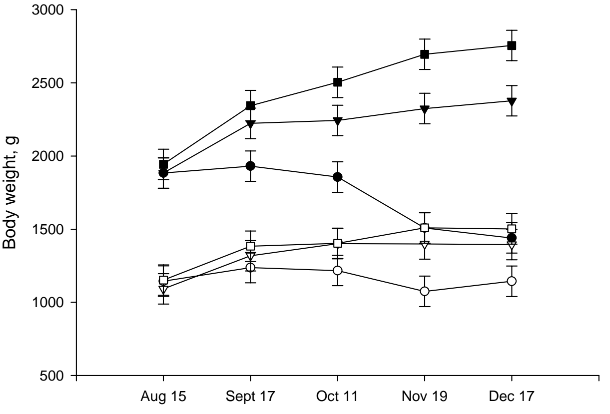
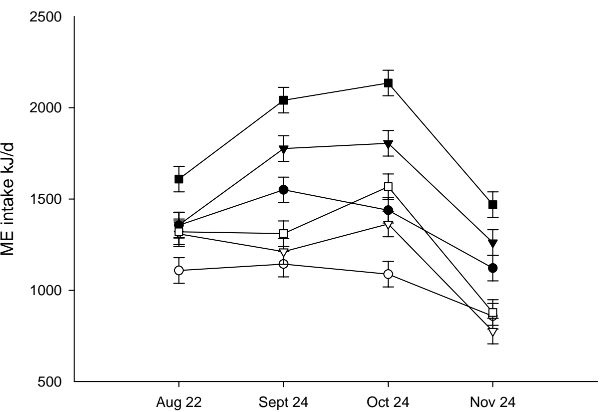


Figure 2.
Daily metabolizable energy intake of juvenile male and female mink in different feeding regime groups based on Recommended Dietary Allowance. The males are presented with closed and the females with open symbols. The symbols used are as follows: circle 80%RDA, triangle 100%RDA, and square 120%RDA. Presented are lsmeans∀SEM. Planned comparisons: August females: 80%RDA-100%RDA P=0.040, 80%RDA-120%RDA P=0.030, males 80%RDA-120%RDA P=0.010, 100%RDA-120%RDA P=0.011; September males: all groups P#0.021; October females: all groups P<0.05, males: all groups P<0.001; November males: 80%RDA-120%RDA P<0.001, 100%RDA-120%RDA P=0.033.



As expected, increasing the daily energy intake resulted in heavier body weights in both juvenile male and female mink, whereas restricted feeding reduced weight gain. The mink is very responsive to changes in energy supply and has a propensity for seasonal fatness (Tauson & Forsberg, 2002). Short-term variations are seen in mink body condition particularly during winter when cold weather induces reduced feed intake and diminished locomotor activity (Korhonen & Niemelä, 1993). It is likely that the colder weather during the latter part of the current experiment had more of an impact on the feed intake and subsequently body weight maintenance of the female mink resulting in an overall less gain in body condition. This may be also related to the more pronounced heat loss in the females due to their smaller body size. The only exception to this were the male mink in the 80%RDA regime, where rapid loss in body weight was apparent from October to December. It is to be noted, although no detailed behavioural observations were carried out, that these

males were also physically very active and were often observed running back and forth in the pen when approached by humans. It is interesting to note that the males in this group also had the highest levels of blood urea nitrogen (17.94 mmol l^{-1}) and the lowest levels of calcium (2.21mmol l^{-1}) in comparison to all other mink (BUN: range 9.99-12.11 mmol l^{-1}, SEM=1.21, Ca: range 2.35-2.45 mmol l^{-1}, SEM=0.045)(P<0.05).
In the end of the experiment, when organ and tissue samples were taken, it was notable that the 80%RDA males had hardly any subcutaneous body fat and the internal fat depots present had the appearance of brown adipose tissue. This may suggest cold acclimation due to reduced body insulation. The 100%RDA and the 120%RDA regime males, on the other hand, had well developed body fat depots, and the latter exhibited excessive visceral adiposity. The observed differences in the females were much less apparent and were thus in line with the less extreme differences in feed consumption and body weight accumulation (Figures 1 and 2). Similar findings have been reported earlier by Korhonen and Niemelä (1998) where *ad libitum* and restricted feeding of male mink resulted in more pronounced differences among test groups in comparison to those seen in the females.

Body condition score
At the start of the experiment, no differences existed in the body condition among the mink in the different feeding intensity groups (P=0.668), however, the males tended to be scored heavy more often (8/15), while all the females scored ideal (P=0.002). In October, 9/10 mink in the 80%RDA group and 7/10 in the 100%RDA group received a BCS 3 (ideal), while in the 120%RDA group 7/10 animals scored 4-5 (heavy-obese) (P=0.040). In December, in the 100%RDA group 6/10 mink scored 3, while in the 80%RDA group 7/10 mink received a BCS 2 (thin), and in the 120%RDA group 8/10 mink had a BCS 4-5 (P<0.001).

Glycemic control
There were no differences in the blood glucose values between the treatment groups at the start of the experiment. The average values for the males and females were found to be 5.33 and 5.08 mmol l^{-1} (SEM=0.30). Throughout the experiment, the only statistically significant effect was that of the experimental treatment (P=0.013). In October, the blood glucose values measured were higher in the females in the 120%RDA group (6.04 mmol l^{-1}) compared to those in the 100%RDA group (4.48 mmol l^{-1}; SEM 0.52, P=0.039) but not different from the 80%RDA group (4.84 mmol l^{-1}; P=0.110). The male groups did not differ from each other at this time point. The final blood glucose levels measured in December (Figure 3) of all mink in the 120%RDA group were on average higher (6.59 mmol l^{-1}) in comparison to the 80%RDA (5.21 mmol l^{-1}) and the 100%RDA feeding intensity groups (4.95 mmol l^{-1}) (SEM=0.37, P#0.01). There was no significant sex effect at this time. None of the mink exhibited glucosuria during the experiment. In December, the males in the 120%RDA feeding regime group showed pronounced hyperinsulinemia (2.06 ng ml^{-1}, SEM=0.164, P#0.046) in comparison to the rest of the mink (range 1.17-1.51 ng ml^{-1})(Figure 4). There was no effect of the feeding regime on the post-prandial free fatty acid values (males 388.1 µmol ml^{-1}, females 372.4 µmol ml^{-1}, SEM= 38.3), but a trend among the regime groups in the triacylglycerol concentrations (males: 80%RDA 40.2 µmol ml^{-1}, 100%RDA 60.3 µmol ml^{-1}, 120%RDA 65.7 µmol ml^{-1}, females: 80%RDA 40.9 µmol ml^{-1}, 100%RDA 80.2 µmol ml^{-1}, 120%RDA 34.5 µmol ml^{-1}SEM=10.5) (P=0.052).
Since the juvenile female mink achieve their adult body size earlier (Hansen et al., 1991), it is likely that the autumnal body fat deposition may have interfered with glucose disposal in the female mink earlier than what was observed in the males, as shown in the 120%RDA group in October. Based on the documented higher activities of glucose-6-phosphatase and pyruvate kinase in the female mink (S rensen et al., 1995), they may also show more pronounced gluconeogenesis and glycolysis. This may result in higher blood glucose levels, which may be further augmented by handling stress (Clausen et al., 1999). Later in the fall, the females gained relatively less body condition following the 100%RDA and the 120%RDA feeding regimes in comparison to the males in the respective regimes.

Figure 3.
Final blood glucose concentrations of juvenile male and female mink in the different feeding regime groups based on Recommended Dietary Allowance (RDA). The males are presented with black and the females with white bars. Presented are lsmeans∀SEM. Planned comparisons: males: 80%RDA-120%RDA P=0.015, 100%RDA-120%RDA P=0.008; females: 100%RDA-120%RDA P=0.099.

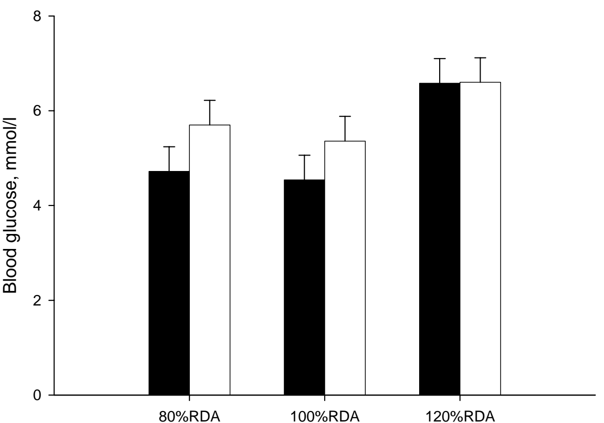
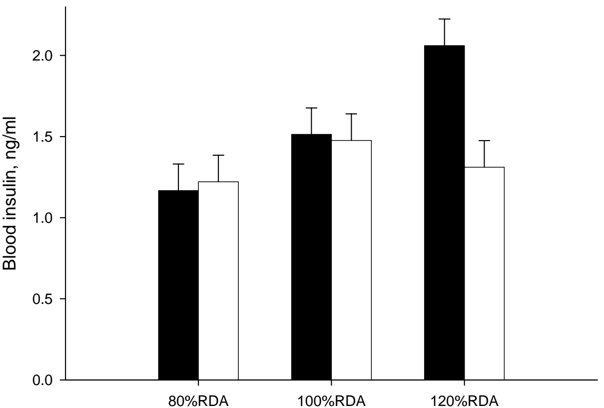


Figure 4.
Final blood insulin concentrations of juvenile male and female mink in the different feeding regime groups based on Recommended Dietary Allowance (RDA). The males are presented with black and the females with white bars. Presented are lsmeans∀SEM. Planned comparisons: males 120%RDA – other groups P<0.05.



Therefore, although the overfeeding and associated heavy-obese body condition did result in hyperglycemia in the females also, it did not cause abnormally high insulin levels as was observed in the heavy-obese males. Based on body composition analyses using tritium-labeled water, juvenile male mink have more lean body mass than female mink in August and in November (Boudreau, 2004). This is likely to result in better clearance of glucose from the blood stream by the muscle tissue and may therefore delay the development of hyperglycemia in response to autumnal fattening. However, later in the fall the males, due to being better able to cope with the cold weather due to their larger body size, may not lose much body fat, and may then show more pronounced signs of compromised glycemic regulation. This is perhaps further compromised by the cold weather inducing longer periods of physical inactivity.

The adipose tissue is an active endocrine and secretory organ that performs a vital role of buffering fluxes of fatty acids in the circulation. When this buffering capacity has been exceeded, such as in obesity, deposition of fat in other tissues interferes with insulin-mediated glucose disposal and leads to insulin resistance (Frayn, 2001). It is important to note that the increased availability of non-esterified fatty acids is also a potent stimulus for hepatic glucose production (Frayn 2001). It has recently been suggested that uncontrollable gluconeogenesis during lactation may cause hyperglycemia (Fink & Børsting, 2002), a key clinical finding in females suffering from nursing sickness (Wamberg et al., 1992). In the current study, contrary to what was expected, the post-prandial triacylglycerol or free fatty acid concentrations did not differ between the treatment groups. Therefore this mechanism may not explain the development of insulin resistance in the mink. Most recently, Dandona et al. (2004) have proposed that the link between obesity, insulin resistance and diabetes is the inflammatory response. Obesity (chronic overnutrition) and hyperglycemia are both classified as pro-inflammatory states, which via inflammatory mechanisms would result in the inhibition of insulin signaling and the development of insulin resistance (Dandona et al. 2004). It is evident from the results of this study that the macro nutrient supply and the body condition of the mink significantly impact glycemic regulation. The hyperinsulinemia observed in the male mink also suggests that autumnal fattening may lead

to the development of insulin resistance. This in turn may be a key predisposing factor to the later development of nursing sickness, the etiology of which is strongly linked to poor glycemic control.

Conclusions

Increasing daily energy intake by a higher feeding intensity resulted in heavier body weights in juvenile male and female mink, whereas restricted feeding resulted in lower weight gain (females) or loss of body condition (males). Chronic overnutrition and the development of obesity during the fall was associated with elevated blood glucose concentrations in both male and female mink and hyperinsulinemia in the males suggesting insulin resistance. It is surmised that autumnal fattening may be a significant predisposing factor to the development of nursing sickness, the etiology of which is strongly linked to impaired blood sugar regulation.

References

Boudreau, D. J. 2004. Influence of poultry based diets on mink *Mustela vison* pelt quality and skin and hair histology. M.Sc. Thesis. Nova Scotia Agricultural College, Truro, Nova Scotia, and Dalhousie University, Halifax, Nova Scotia. Under review.

Canadian Council on Animal Care 1993. Guide to the care and use of experimental animals. Ottawa, ON.

Clausen, T. N., Hansen, O. & Wamberg, S. 1999. Plasma cortisol concentration as an indicator of stress in mink dams at weaning. Scientifur 23, 271-273.

Dandona, P., Aljada, A. & Bandyopadhyay, A. 2004. Inflammation: the link between insulin resistance, obesity and diabetes. Trends in Immunology 25(1), 4-7.

Fink, R. & Børsting, C. F. 2002. Quantitative glucose metabolism in lactating mink (*Mustela vison*) - effects of dietary levels of protein, fat and carbohydrates. Acta Agric. Scand., Sect. A. Anim. Sci. 52, 34-42.

Frayn, K. N. 2001. Adipose tissue and the insulin resistance syndrome. Proceedings of the Nutrition Society 60, 375-380.

Hand, M.S., Thatcher, C.D., Remillard, R.L., & Roudebush, P. 2000. (Eds.) Small Animal Clinical Nutrition. 4th edition. Mark Morris Institute, Walsworth Publishing Company, Marceline.

Hansen, N. E., Finne, L., Skrede, A. & Tauson, A.-H. 1991. Energiforsyningen hos mink og r v. Nordiske Jorsbrugsforskeres Forening, NJF-Utredning/Rapport Nr. 63. DSR Forlag, Landbohojskolen. Copenhagen, Denmark.

Hoenig, M. & Ferguson, D. C. 1989. Diabetes mellitus in dog and cats. Small Anim. Pract. 19, 13-14.

Korhonen, H. & Niemelä, P. 1993. Winter energetics and feeding activities in the male mink. Scientifur 17, 137-142.

Korhonen, H. & Niemelä, P. 1998. Effect of *ad libitum* and restrictive feeding on seasonal weight changes in captive minks (*Mustela vison*). J. Anim. Physiol. a. Anim. Nutr. 79, 269-280.

Littell, R.C., Henry, P.R. & Ammerman, C.B. 1998. Statistical analysis of repeated measures data using SAS Procedure. J. Anim. Sci. 76, 1216-1231.

National Research Council 1982. Nutrient Requirements of Mink and Foxes. National Academy Press. Washington, DC.Plotnick, A. N. & Greco, D. S. 1995. Diagnosis of diabetes mellitus in dogs and cats. Small Anim. Pract. 25, 563-570.

Rouvinen-Watt, K. 2003. Nursing sickness in the mink (*Mustela vison*) - a metabolic mystery or a familiar foe? Review Article. Can. J. Vet. Res. 67, 161-168.

Rouvinen-Watt, K. & Armstrong, D. 2002. Body condition scoring of mink using a five-point scale. Nova Scotia Agricultural College, Canadian Center for Fur Animal Research, Truro, NS.

Sørensen, P.G., Petersen, I.M., & Sand, O. 1995. Activities of carbohydrate and amino acid metabolizing enzymes from liver of mink (*Mustela vison*) and preliminary observations on steady state kinetics of the enzymes. Comp. Biochem. Physiol.112B, 59-64.

Tauson, A.H. & Forsberg, M. 2002. Body weight changes are clearly reflected in plasma concentrations of leptin in female mink (*Mustela vison*). J. Nutr. 87, 101.

Wamberg, S., Clausen, T. N., Olesen, C. R. & Hansen, O. 1992. Nursing sickness in lactating mink (*Mustela vison*). II Pathophysiology and changes in body fluid composition. Can. J. Vet. Res. 56, 95-101. .

Acknowledgments

We thank the staff of the Canadian Centre for Fur Animal Research, Ms. Amber Hynes and other members of the research team for technical assistance. Funding for this project was provided by the Canada Mink Breeders Association, the Mink Farmers Research Foundation and the Heger Company, USA.

III – 8 RP

The effect of *ad libitum* and restricted feeding on growth curves and growth rate curves in mink selection lines

V.H. Nielsen, S.H. Møller**, B.K. Hansen* and P. Berg**

**Department of Animal Breeding and Genetics and ** Department of Animal Health and Welfare, Danish Institute of Agricultural Sciences, Research Centre Foulum, 8830 Tjele, Denmark.*

E-mail: vivih.nielsen@agrsci.dk

Abstract

The effect of *ad libitum* feeding (AL), restricted feeding (RF) and farm feeding (FF) on growth curves and growth rate curves is studied in lines of mink destined for selection for high November weight. The results show that the growth curves can be described by a fourth degree polynomial specific to line and sex and significant differences are found between the AL-line and the RF-line ($P<0.0001$ for both November weight and overall growth rate). Body weight and growth rate are reduced in the RF-line compared to the AL-line. This suggests a moderate to low genetic correlation between growth on the two feeding regimes that is the basis for a differentiated response to selection.

Introduction

Selection for growth on restricted feeding is assumed to improve feed efficiency as found in mice (Hetzel & Nicholas, 1986; McPhee & Trappett, 1987; Urrutia & Hayes, 1988). Technological progress in management of feeding has made recording of feed allowance possible in mink production and made both *ad libitum* and restricted feeding possible. Thus, the use of restricted feeding to improve feed efficiency can be studied in mink as well. The way of presenting the problem is one of genotype-environment interaction.

The effect of the restricted feeding has to be surveyed regularly during the growth period to secure a current reduction in growth. Successive observations of weights from an animal are correlated. A way of dealing with records from a growth curve is to analyze data using a random regression model (Andersen & Pedersen, 1996). This study reports the growth patterns in lines of mink on *ad libitum* feeding (AL), restricted feeding (RF) and farm feeding (FF) in a selection experiment in the first generation before selection is performed. Weight is modelled as a function of age and a function for growth rate is obtained as first derivative. Furthermore, the average curves are estimated for growth and growth rate.

Materials and methods

Animals: The animals used for the present feeding experiment are the base population in a selection experiment. Three lines were established for the selection experiment. Line FF is a control line. Line AL and RF are lines selected for high November weight on *ad libitum* and restricted feeding, respectively. All three lines were established by crossing two previous scanbrown mink selection lines. The one line was selected exclusively for high November weight, while in the other line, male mink were selected for high November weight and female mink were selected for high litter size. Line FF, AL and RF were constructed to be as genetically similar as possible. Each line is maintained by 100 female mink. For the feeding experiment recordings from 381, 363 and 366 animals from line FF, AL and RF, respectively are used in the data analysis.

Feeding: The animals were set out and weighed across three days in late June and the test feeding was commenced within a week and at the same day for all lines. A standard feed kitchen diet was used. A detailed description of the feeding procedure is given by Møller *et al*. (2004). Line FF was farm fed according to normal farm procedure. Line Al was fed *ad libitum* and line RF was kept under a restrictive feeding regime and fed 90% of the amount of feed offered to the control line. *Ad libitum* feeding in line AL was obtained by individual feeding at cage level using a computerized feeding machine regulated by a Palm Pilot. The feeding machine was used for feeding line FF and RF as well.

Weights: Individual weights were recorded in all lines at three weeks intervals from the time the animals

were set out in pairs (from 26 June to 1 July) until pelting. Eight weights were recorded for each animal.

Models: In the analysis of growth curves it has to be considered that observations from an animal are correlated. Thus, a random regression model with orthogonal Legendre polynomials was used. The model assumes that the growth curve for each animal is described by a fourth degree polynomial and random animal deviations following a second degree polynomial. The model is:

$$W_{ia} = .(a_{ls}+A_i)+(c_{ls}+C_i)*lgc_1+(g_{ls}+G_i)*lgc_2+h_{ls}*lgc_3+ j_{ls}*lgc_4+e_{ia} \quad (1)$$

W_{ia} is the weight of animal i at age a. In the model a_{ls}, c_{ls}, g_{ls},, h_{ls} *and* j_{ls} are fixed effect parameters of line and sex. A_i, C_i and G_i describe random regression parameters, describing permanent animal effects, while the deviation from the individual curve is modelled by e_{ia}. (A_i, C_i, G_i) is multivariate normally distributed N(0,V) and the e_{ia} are independent N(0,σ^2). lgc_1 - lgc_4 are normalized Legendre covariates. A set of covariates is obtained for each age.
The average curves are estimated as:

$$\text{Average}(W_a) = \hat{a}_{ls}+\hat{c}_{ls}*lgc_1+\hat{g}_{ls}*lgc_2+\hat{h}_{ls}*lgc_3+\hat{j}_{ls}*lgc_4 \quad (2)$$

$\hat{a}_{ls}$, ,$\hat{c}_{ls}$, $\hat{g}_{ls}$, $\hat{h}_{ls}$, and $\hat{j}_{ls}$ are the estimates of the parameters.

A function for growth rate is obtained as the first derivative of the function for weight. Thus the model for growth rate of animal i at age a is:

$$GR_{ia} = (c_{ls}+C_i)*lgc_1'+(g_{ls}+G_i)*lgc_2'+h_{ls}*lgc_3'+j_{ls}*lgc_4'. \quad (3)$$

lgc_1' -lgc_4' are the derivatives of lgc_1 - lgc_4. The average growth rate curves are estimated as:

$$\text{Average}(GR_a) = \hat{c}_{ls}*lgc_1'+\hat{g}_{ls}*lgc_2'+\hat{h}_{ls}*lgc_3'+\hat{j}_{ls}*lgc_4' \quad (4)$$

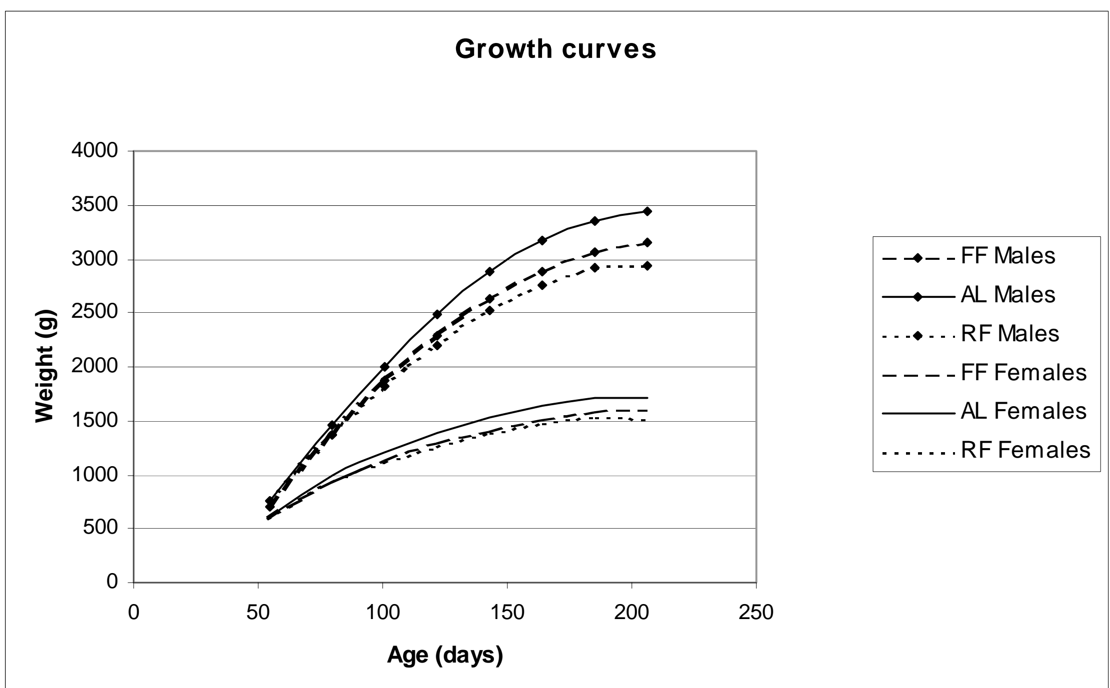
Results
The estimates of the parameters obtained from analysis of model 1 have no biological interpretation but serve in fitting the growth curves. The estimates of the parameters are given in Table 1. The growth curves are shown in Fig. 1 and the growth rate curves are shown in Fig. 2.

Growth: The weight of the females was less than the weight of males at the start of the test (P<0.0001) and a significant difference between line-sex groups was found at all times of weight recordings (P<0.0001). Comparison of lines within sex showed a significant difference between the AL-males and the RF-males already at the first weighing on test at an age of about 84 days (P=0.01). The difference between the respective females at the same time was also large but not significant (P=0.05). Thus, the effect on growth

Table 1. Fixed effect parameters (±S.E.) for growth curves estimated from the random regression model. Feeding regimes are: farm fed (FF), *ad libitum* (AL) and restricted feeding (RF).

Feeding regime	FF		AL		RF	
Parameter	Males	Females	Males	Females	Males	Females
	2319± 15	1290± 15	2522± 16	1385± 15	2224± 15	1258± 15
c	1102± 11	435± 11	1222± 11	484± 11	996± 11	401± 11
g	-269± 5	-139± 5	-296± 5	-159± 5	-268± 5	-152± 5
h	-7± 3	15± 3	-29± 3	9± 3	-22± 3	4± 3
j	6± 3	-21± 3	13± 4	-15± 4	-11± 4	-28± 4

Figure 1. Growth curves from 55 to 206 days of age in the line on farm feeding (FF), *ad libitum* feeding (AL) and restricted feeding (RF).



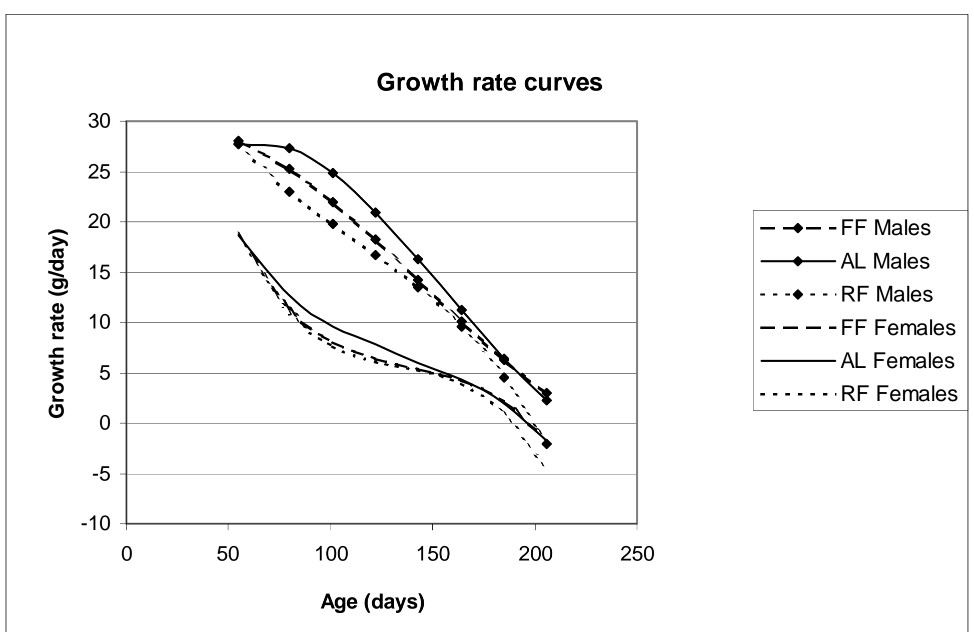
was obtained immediately after start of the test feeding. At the following recordings all differences between lines within sex were significantly different with one exception. FF females and RF females never diverged significantly ($P>0.08$).

Growth rate: An overall significant difference between line-sex groups was also found for growth rate in all periods ($P<0.0001$). The overall growth rate was considerably smaller in females than in males ($P<0.001$) and from 50 to 100 days of age the decrease in growth rate was larger in females (Fig. 2). Until around 105 days of age, comparison showed large and often significant differences between lines except for the difference between the FF females and the RF females. In the following periods the difference between these two lines was only significantly different for both males and females in the last part of the growth period ($P<0.0001$ for males and $P=0.008$ for females). This difference was probably obtained due to an intentionally larger feed restriction with the purpose of an increased reduction in growth in the RF line. Comparison of line AL and RF showed that growth rate generally was significantly different for both males and females ($P<0.04$ for both males and females). However, between 148 and 168 days of age the restriction of line RF was not sufficient to create a significant difference between these lines ($P=0.18$ for males and $P=0.57$ for females).

Discussion and conclusion

Results from selection experiments have shown that growth on a restricted diet or a diet with a suboptimal feed composition compared with growth on *ad libitum* feeding has a rather different genetic basis (Hetzel & Nicholas, 1986; McPhee & Trappett, 1987; Nielsen & Andersen, 1987; Urrutia & Hayes, 1988). This requires that the restricted feeding causes a reduction in growth. The results of the present feeding experiment show significant differences in weight development and growth rate during the growth period between the AL and RF line. Thus the results suggest that the two feeding regimes creates a basis for a differentiated response to selection.

Figure 2. Growth rate from 55 to 206 days of age in the line on farm feeding (FF), *ad libitum* feeding (AL) and restricted feeding (RF).



References

Andersen, S. & Pedersen, B. (1996). Growth and food intake curves for group-housed gilts and castrated male pigs. Animal Science 63, 457-464.

Hetzel, D.J.S. & Nicholas, F.W. (1986). Growth, efficiency and body composition of mice selected for post-weaning weight gain on ad libitum or restricted feeding. Genetical Research. Camb. 48, 101-9.

McPhee, C.P. & Trappett, P.C. (1987). Growth and body composition changes in mice selected for high post-weaning weight gain on two levels of feeding. Theoretical and Applied Genetics 73, 92 6-931.

Møller, S.H., Nielsen, V.H. & Hansen, B.K. (2004). Individual *ad libitum* feeding of male + female pairs of mink kits during the growth period increases feed intake, weight gain and feed efficiency. VIII International Scientific Congress in Fur Animal Production (Publication).

Nielsen, B.V.H. & Andersen, S. (1987). Selection for growth on normal and reduced protein diets in mice. I Direct and correlated responses for growth. Genetical Research Camb. 50, 7-15.

Urrutia, M.S. & Hayes, J.F. (1988). Selection for weight gain in mice at two ages and under *ad libitum* and restricted feeding. Theoretical and Applied Genetics. 75, 424-431.

III – 9 P

Sodium bisulfate as a mink food preservative

William L. Leoschke, Ph.D.
Valparaiso University
Valparaiso, Indiana 46383, U.S.A.
Gina.Doneske@valpo.edu

Introduction

For half a century, phosphoric acid (75% feed grade) has been employed in mink ranch diets throughout the world wide fur industry as a feed "on the wire" preservative and as an effective urinary acidifier prophylactic medicinal program for minimizing the formation of struvite urinary calculi in mink and fox diets at the 2%, Leoschke, 1956; and 2.5%, Leoschke, 1996; levels on a dehydrated basis.

In recent years, there has been public concern in the United States and Europe about phosphate pollution of the environment. Sodium bisulfate is an alternative mink feed and urine acidifier which has been shown to be safe and effective for modern mink nutrition in field observations in the United States and Canada in recent years.

It is of interest to note that sodium bisulfate has been shown to be a useful urine acidifier for the prevention of struvite calculi in cats for a number of years, Kneuven, 2000. It has also been used in Europe as a silage preservative for cattle feed for many years.

Materials and Methods

The pKa (the chemists' mathematical basis for designating the relative acidity of a specific acid) of phosphoric acid, 2.16 and bisulfate anion, 1.99 are very similar and thus their employment as a mink feed preservative at equal levels of acid concentration should in theory yield about the same final pH of the feed as noted in experimental data involving a fish slurry provided by Jones-Hamilton, Co., Walbridge, Ohio. The product marketed by this company contains about 93% sodium bisulfate and 7% disodium sulfate and thus contains about 75% bisulfate anion similar to the level of phosphoric acid provided in commercial phosphoric acid (75% feed grade).

Results

All factors considered, it does appear that in terms of field observations of United States mink ranchers employing sodium bisulfate that this acid resource is not quite as effective as a mink feedstuff preservation chemical or as an acidifier for mink feed "on the wire" as phosphoric acid. Rancher observations of Zimbal, 1998; and Brown, 2000; have indicated that sodium bisulfate was not as effective as phosphoric acid (75% feed grade) for the preservation of raw egg product at room temperature. In the case of the Walter Brown Mink Ranch, the employment of 3% phosphoric acid (75% feed grade) with his raw egg resource achieved a raw egg mixture with a pH of 3.5 and zero bacteria population after 3-5 days storage.

The employment of sodium bisulfate at a level of 2.0% yielded a raw egg mixture pH of 3.6 but a reduced effect on lowering bacterial populations. Observations of Durant, 1999; on the urinary pH of the mink fed a redi-mix program with 0.8% phosphoric acid (75% feed grade) (equivalent to 2.0% phosphoric acid dehydrated feed basis) or 0.9% sodium bisulfate indicated a higher mink urinary pH with the sodium bisulfate supplementation relative to that with the phosphoric acid addition. It is of interest that Durant employed sodium bisulfate at levels as high as 2.5% (dehydrated basis) in the ready-mix program without any observations of negative effects on feed consumption.

Experimental studies reported by Jones-Hamilton Co. with cats on a pellet program indicated that 0.9% sodium bisulfate yielded an average urinary pH of 6.4 – a urinary pH considered to be effective in minimizing struvite calculi formation in cats and mink. This urinary pH data is similar to studies with mink at the National Research Ranch with sodium bisulfate at a 1% level (dehydrated basis), Michels, 2002. It is of interest to note that the field studies in North America in 2002 involved the employment of sodium bisulfate at the level of 1.5%, dehydrated basis.

Discussion

It is apparent that sodium bisulfate is a useful alternative to phosphoric acid (1) as a mink feed preservative "on the wire" and (2) as an effective urinary acidifier for the prevention of the formation of struvite calculi in the mink – made to those levels

of sodium bisulfate required for the top performance of the mink.

References

Brown, W., 2000. South Dakota Mink Rancher, personal communication.
Durant, G., 1999. Utah Mink Ranchers Redi-Mix Co-Op, personal communication.
Knueven, C., 2000. Sodium bisulfate, a potential new acidifier for the pet food industry. Petfood Industry, June 2000.
Leoschke, W. L., 1956. Phosphoric acid in the mink’s diet. American Fur Breeder, 29(8):18
Leoschke, W.L., 1996. Phosphoric Acid in modern mink and fox nutrition.
Animal Production Review. Polish Society of Animal Production.
Applied Science. Animal Production Reports, 28. Progress in Fur
Animal Science. Nutrition, Patology and Disease. Proceedings from the Vth
Inter. Sci. Congr. in Fur Animal Production, August 21-23, 1996, Warsaw, Poland:77-78.
Michels, M., 2002. National Research Ranch, New Holstein, Wisconsin, unpublished data.
Zimbal, R. 1998. Wisconsin mink rancher, personal communication.

III – 10 RP

The “boiling” phenomenon in formic acid preserved poultry by-products

Øystein Ahlstrøm[1)], John Erik Haugen, [2)], Egil Kjos [3)], and Tom Granli [4)]
1) Department of Animal and Agricultural Sciences, Agricultural University of Norway
P.O. Box 5025, N-1432 Ås, Norway.
2) Norwegian Food Research Institute, Osloveien 1
N-1430 Ås, Norway.
3) Norwegian Fur Breeders’ Association, Laboratory Division, P.O. Box 175, N-0509 Økern, Oslo, Norway,
4) Hydro Formates, Arthur Berbysvei 6, N-3908, Porsgrunn, Norway

Abstract
Formic acid preserved poultry by-products has become an important ingredient in Norwegian fur animal feed. Occasionally this semi-liquid product tends to overrun tanks during transport owing to gas coming from the mass. This so called “boiling” phenomenon causes practical problems cleaning up and sometimes destruction of the product. This study showed that *Clostridia* may be responsible for the significant gas production and that hydrogen gas production derived by *Clostridium* bacteria play an important role in the over-running. The findings suggest that formic acid preserved poultry by-products should be handled with caution, since it may represent a health risk related to the exposure to pathogenic *Clostridia* and secondly, because of the occurrence of hydrogen, which is highly explosive. The use of additives as sodium bisulphite (0,5 %), which may have an inhibitory effect on gas generation can be a future solution to the “boiling” phenomena.

Introduction
In Norway, poultry by-products (viscera, heads, feet) used as ingredient in fur animal feed are preserved with formic acid at pH 3.5-3.8 to prevent bacterial growth.. The preserved product is semi-liquid (app. 25 °C) and is transported in tank trucks to the fur animal feed producers. Occasionally, the mass expands and runs over the tanks during transport and may continue running over after being loaded to storage tanks. Typical signs of this so called “boiling” phenomena, are bubbles and foaming similar to boiling, indicating the occurrence of gas production the mass. The over-running may last for 1-3 days and it appears to terminate by it self. Similar problems with overrunning have been reported with fish silage preserved with formic acid. The economic cost of this phenomenon is related to extra clean-up work and in some cases loss due to destruction of the product.
The main objective of the present experiment was to identify causes for the so-called “boiling” cases, i.e. gas –production during storage and transport of acid stabilised poultry by-products. This was obtained by carrying out small scale laboratory storage trials on freshly preserved raw material in combination with volumetric measurements of gas production, chemical gas analysis and microbial analysis. In addition trials were carried out to test the inhibitory effect of gas-production by a sodium bisulphite, which in earlier studies has shown to have a possible inhibitory effect on gas-production.

Material and methods
Samples
Measurement experiments were performed on raw material from different batches from the slaughter-waste plant at Prior Sør, Rakkestad, Norway. Samples of grinded (20 mm hole plate) poultry by-products were taken before and after addition of formic acid (2-3 %) from the slaughterhouse. Acid was added just a few minutes after slaughter. In addition, acid preserved samples were taken, which had been kept for a few days on the storage tank at the plant. The acid preserved samples represented both stable and unstable samples with regard to extent of gas production.

Chemical contents and hygienic quality characteristic
Protein, fat, ash content, pH, total volatile nitrogen, were determinedin unpreserved and preserved samples. In addition, analysis of organic volatile

compounds using gas-chromatography mass-spectrometry was performed on selected samples. Hydrogen analysis was performed by using a gas chromatography (HP 6890, Supelco, 80/100 mesh mole sieve 5A, 3 m, 1/8”) in combination with a thermal conductivity detector.

Microbiological analysis

The samples were characterised by analysing the microflora under both aerobic and anaerobic conditions. Total viable count, coliform bacteria and fungi were determined using standard methods at Norwegian Fur Breeders’ Association, Laboratory Division. For determination of *Clostridia* blood agar, TSC-medium and boiled meat broth growth media were applied. Catalase test was used to confirm the presence of *Clostridia* like bacteria. In addition, DNA fingerprinting analysis using RAPD techniques was applied on a few samples for the confirmation of *Clostridia* strains. Also microscopy was used for identification of bacteria colonies based on morphology.

Measurement of gas-production

Three Samples (450 g) of formic acid preserved by-products were transferred to 500 ml flasks where silicone tubing were connected through the sealing and led into a water bath and into a water-filled graded burette for volumetric measurement of water displacement during gas production. Corresponding samples (n=3) (450 g) were packed in plastic bags and vacuumated. The samples were stored at room temperature (21°C) for seven days. About 50 g samples were heat shocked (80°C) to kill living microorganismes and to analyse for sporogenous bacteria in the material afterwards. The same experiment with vacuumated bags was repeated including also acid preserved samples added 0.5 % sodium bisulphite.

Statistical treatment

Because of the preliminary character of this study, only average values with standard deviations are presented.

Results and discussion

Chemical contents, hygienic quality characteristic and microbiological analyses

The pH of the starting material varied from 3.3-3.8. Chemical composition data revealed that the by-products had somewhat higher dry matter content before than after acid preservation (Table 1). This difference is probably due to the fact that the fresh samples were taken just after grinding, while the acids preserved were taken from a larger mixed volume. However, the most likely reason is a dilution effect from adding formic acid. Typically, dry matter content is about 30 %, and protein and fat account for approximately 40-50 % each. Ash content is often 2-4 % (10-15 % of DM). High ash levels may challenge the buffer capacity of formic acid by releasing Ca, and by that make the pH increase above 4.0, which is a critical level to stay below to avoid bacterial growth. The TVN values were low, showing that the protein degradation had been moderate. However, the hygienic quality of the fresh, unpreserved product revealed considerable bacterial counts, up to log

Table 1. Dry matter, protein, fat, ash, total volatile N, total bacterial count, coliform bacteria and fungus in poultry by-products before and acid preservation (n=6). Standard deviation in parentheses

	Fresh	Formic acid preserved
Dry matter (%)	31.4 (2.2)	27.4 (2.2)
Protein (%)	14.2 (1.3)	12.1 (1.1)
Fat (%)	13.0 (2.8)	10.9 (1.7)
Ash (%)	3.2 (0.8)	2.2 (0.3)
TVN (mg/100g)	8.7 (3.4)	7.5 (0.8)
PH	6.62 (0.19)	3.60 (0.22)
Total viable count (logN)	6.5 (6.4)	3.2 (3.2)
Coli bacteria (logN)	5.3 (5.1)	<1
Fungus (logN)	3.7 (3.8)	<1

6.84 (average log 6.5), but the variation between samples were large. The efficiency of formic acid preservation was clear. Adding 2-3 % formic acid (85%) improved the hygienic quality by killing of microorganisms to a considerable extent. Coli bacteria and fungus were brought to a very low level. During the storage experiments, growth of yeast could be observed by microscopy on the surface of the flask samples, due to the presence of air, and *Clostridium* like bacteria further down in the mass. No yeast growth could be detected under the anaerobic conditions prevailing in the bag samples. Microbial analysis of both stable and unstable freshly acid preserved raw material before storage at room temperature revealed the presence of only *Clostridia* under anaerobic conditions, which was indicated by microscopy and catalyse test. Colony morphology suggested the presence of *Clostridium perfringens* and possibly *Clostridium tetani*. *Clostridium perfringens* was positively confirmed by the RAPD method, but the second strain could not be confirmed uniquely. The occurrence of *Clostridia* is in agreement with previous findings in poultry meat (Kaldhusdal et al. 2001). During the storage experiment, an increase in pH could be observed up to 4.5 over one week. The pH and water activity are crucial growth limiting factors for *Clostridia*, having an optimum around pH 7, and DM preference up to 30 % (McDonald, 1981). Water activity in our case around the limit, but at the acidic conditions used, it is little likely that these bacteria should be able to grow. However, the raw material represents a rather heterogeneous mass, with possibility for local pockets with favourable conditions that may allow the growth of *Clostridia*. On the other hand, growth of some *Clostridia* strains may occur down to pH 4.1 (McDonald, 1981), which is at pH levels found during our storage experiments.
Experience from practice indicates that Norwegian produced poultry by-products might have higher levels of *Clostridia* than in by-products from Denmark and Finland. The reason for this difference is not clear, but it could be owing to different feeding regimes in the production of poultry.

Measurement of gas production
Incubation of the formic acid preserved poultry by-products in flasks and vacuum bags produced considerable amounts of gas. In the bags one could see clearly bubbles coming out of the product. By repeated experiments on different batches, total gas production rates under anaerobic conditions varied from 500 up to 9000 ml/kg/day.

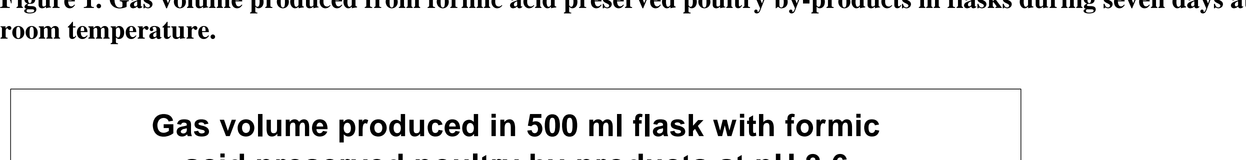


Figure 1. Gas volume produced from formic acid preserved poultry by-products in flasks during seven days at room temperature.

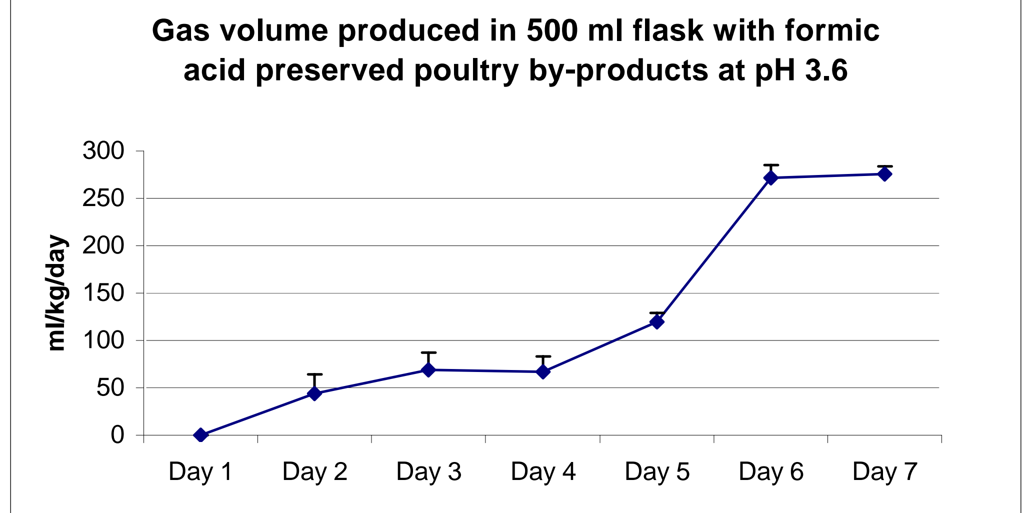


Figure 2. Gas volume produced from formic acid preserved poultry by-products in vaccum plastic bags during seven days at room temperature

Gas volume produced in vacuum plastic bags with formic acid preserved poultry by-products at pH 3.6

In aerobic conditions the rates were 10- 30 times lower compared with the anaerobic conditions, varying from 50-250 ml/kg/day. The rates increased exponentially with time resembling a bacterial growth curve as shown for two cases (Figure 1 and 2). Extrapolation of the observed gas production rates under anaerobic conditions to tank load dimensions of real industrial conditions, are in agreement with the expansion volumes observed in practice.

Analyses of hydrogen gas and volatile compounds

Gas from the flask contained small concentrations of hydrogen gas after four days, but during the last three days the hydrogen concentration in the flasks increased 3-4 times. In the anaerobic bags the hydrogen concentrations were even 2-3 times higher than in the flasks after 7 days seven. Since *Clostridia* may consume formate as substrate under anaerobic conditions, producing hydrogen and carbon dioxide, this is the most likely explanation for the detected hydrogen. After heat-shocking the samples, still a considerable hydrogen production was detected, indicating germination and regrowth of *Clostridia.* Results from the GC/MS analysis showed that the major compounds, besides from formate, were dominated by typical volatile secondary metabolites, generated by *Clostridia*, which is another evidence for the dominance of these strains in the sample. In contrast to our result, Urlings et al. (1988) found that *Lactobacilli*, *Enterobacteriaceae* and *Mesohilic aerobic bacteria* were considerably more dominating than *Clostridia* in a mink feed mainly based on poultry by-products. However, in their study no addition of organic acids that could have had bactercidal effect was applied. In our experiment, addition of formic acid had probably changed the growth and survival conditions in favour of *Clostridia*.

The inhibitory effect of sodium bisulphite

Repeated storage experiments with and without addition of sodium bisulphite indicated no consistent effect (results not presented). There was a tendency that in cases of low rates of gas production there could not be demonstrated any effect by adding this salt. However, at substantially higher rates of gas production, there was a positive effect by adding sodium bisulphite, suggesting an inhibitory effect on gas production. The reason for the findings at low gas production rates may be due to the fact that these were cases which could not be considered similar to the "boiling" phenomena. Urlings et al. (1988) observed reverse effects on bacterial growth when adding 0.1 % sodium

metabisulphite to poultry by-products. The reason for this difference to our study is probably that we used a higher concentration (0.5 %) of sodium bisulphite.
Sodium bisulphite is commonly used for preservation in fish silage in Denmark and for preservation of poultry by-products in The Netherlands. This year’s practical experience from Norway, indicates that sodium bisulphite (0.2 %) may have effect against “boiling” of formic acid preserved poultry by-products, but more information is provided. One negative factor to be aware of when using feed ingredients preserved with sodium bisulphite is that it destroys thiamine as discussed by Jensen & Jørgensen (1975). Thiamine deficiency problems is, however, unlikely since thiamine supplementation to fur animal feed is much higher than the requirement.

Conclusions

Results from this experiment, show strong evidence that *Clostridia* may be responsible for the significant gas production occurring in acid preserved poultry waste. Accordingly, it may be concluded that hydrogen gas production derived by *Clostridium* bacteria play an important role in the over-running, the so called “boiling” phenomena, of acid preserved poultry slaughter by-products. The findings suggest that poultry slaughter by-products should be handled with care, since it may represent a health risk during treatment and transportation related to the exposure to pathogenic *Clostridia* and secondly, because of the occurrence of significant amounts of hydrogen, which is highly explosive. The use of additives as sodium bisulphite, which may have an inhibitory effect on gas generation can be a future solution to the “boiling” phenomena encountered in formate preserved poultry by-products.

Acknowledgements

Norwegian Fur Breeders’ Association is thanked for financial support of this project. Janina Berg and Brith Pedersen are acknowledged for their assistance with the microbial analysis.

References

Kaldhusdal, M., Schneitz, C., Hofshagen, M. and Skjerve, E. (2001). Reduced Incidence of Clostridium perfringens-Associated Lesions and Improved Performance in Broiler Chickens Treated with Normal Intestinal Bacteria from Adult Fowl. Avian Diseases, 45, 149-156.

McDonald, P. 1981. Clostridia. In: McDonald, P. (Ed.) The biochemistry of silage. Wiley & Sons, Chichester, ISBN 0 0471 27965 X, p. 77-90.

Jensen., M. Jørgensen, G. 1975. Fremstilling og anvendelse af fiskeensilage specielt til mink. Beretning fra Statens Husdyrbrugsforsøk, 427, 79 p.

Urlings, B.A.P., Bijker, P.G.H., van Oostrom C.H.C. 1988. Short time preservation of mink feed by 0.1 % sodium metabisulfite and low temperature. Proc IV International Cong in Fur Animal Production, Toronto, Canada, 298-306

III – 12 P

The importance of protein for young minks fed with dry food

Nikolai E. Kulikov, Nikolai A. Balakirev
V. Afanasiev Research Institute of Fur Bearing Animals and Rabbits
Ramensky District, Moscow Region, Rodniki 140143, Russia
E-mail: NIIPZK@orc.ru

Abstract
The aim of this research is to determine the main parameters of nitrous exchange in young pastel minks.
The mink were fed only with dry full ration granulated feed. The results of 4 balance experiments held in June, September and November (48 minks) were used. Calculations were made using the method of extrapolation by the equation of regression y= bx + a. Endogen urinal nitrate turned out to be 1,0-1,1 per kg of metabolic mass ($W^{0,75}$). The level of faecal metabolic nitrate was 0,258 g in 100 g of eaten dry food.
It was determined that to support zero balance of nitrate, young minks need 30,2 g of protein per kg of live weight in July and 13,0-14,0 g during September to November.

Introduction
It was decided to feed animals according to the norms of exchange energy and digestive protein to increase productivity and economical effictiveness of mink breeding.
This norms had been worked out and used successfully in minks fed with wet fodder.
For rational full ration granulated feed usage the norms have to be defined more precisely.
Earlier we had detected the norms of nitrogen that have to be lost in young minks with full dry ration in period from July till November (Kulikov, 2001).
Endogen nitrogen that is being excreted with urine in all periods 1,0 – 1,1 g per 1 kg. of exchanging weight ($W^{0,75}$). The exchanging nitrogen of faeces is 0,258 g per 100 g eaten dry fodder.
The norms of exchanging energy (EE) and final live weight for young minks of different ages (June - November) were calculated (Kulikov, 2002).
Considerable scientific and practical interest for us is in researching of young minks digestive protein need.

Materials and Methods
The experiments were held in V. Afanasiev research Institute of Fur Bearing Animals and Rabbits "Rodniki", Moscow region, Russia.
From July until slaughtering isolated young minks were fed Only with dry full ration granulated mixed fodder and had free access to water. Granules maintained fish flower, extruded grains, melted oil, stern barm, dry milk, sunflower oil cake, bone flower, mineral and vitamin additions in different ratio.
On 100 kKl EE digestive g.: protein – 9,00; fat – 4,35; carbohydrate – 4,70. Periodically mails were put in special cages that allow calculate amount of eaten food, excreted faeces and urine. Preliminary adapting period lasted for 3 days and registration for 4 days. Analyses of eaten food, excreted faeces and urine let us calculate a nutrient balance and ability to be digested it in minks.
The level of nitrogen sediment in the minks body in different periods of growth was calculated from practically accepted nitrogen and excreted with faeces and urine.
According to the linear regression equation y = bx + a it was calculated the dependence between sedimented (x) and eaten(y) nitrogen.
During the extrapolation to the zero mark of nitrogen sedimentation in the body(x) the equation shows needed nitrogen amount (protein = Nx6,25) to supply zero balance(taking into account exchanging and endogenous nitrogen).

Results of the research
In all it was studied nitrogen balance in 40 minks with the age of 3, 5 and 7 months.
Nitrogen balance data were statistically worked up. According to the results it was calculated the connection between sedimented and eaten(digested) nitrogen amount (Table 1).

Table 1 Season variation of nitrogen sedimentation in young minks

Indexes	Growing period		
	July	September	November
N of minks	12	9	19
Live weight, g	1032± 35	1460± 59	1875± 35
X, sedimented nitrogen g/animal/day	0,933± 0,382	1,902± 0,442	0,861 ± 0,089
У,eaten nitrogen g/animal/day	5,567± 0,445	5,596± 10,661	5,653± 0,311
Coefficient “в”	0,682	1,355	1,575
Coefficient “a”` g/animal/day	4,989	3,019	4,298
Correlation coefficient, r	0,586	0,906	0,4 –51
P<	0,05	0,001	0,05

We have got following data: July $y = 0,682x + 4,989$; September $y = 1,355x + 3,019$; November $y = 1,575x + 4,298$. Correlation coefficient is true ($P<0,001 – 0,05$).
In experiment animals got various amount of food with different chemical composition, in general actually eaten nitrogen is 5,60 – 5,67 g/head/day.
However it’s usage for sedimentation differs a lot: 16,5 % in July; 34,0% in September and 15,2% in November. Thus on 1 g of sedimented nitrogen minks need 5,67; 4,37 and 5,87 g digestive nitrogen per a head per day in July, September and November.
Estimated digestive protein (N x 6,25) for zero balance is 30g in July, 13 – 14 in September – November (on 1 kg of life weight) with indispensable optimal fat-carbohydrate ratio and EE entry.
It is known that protein assimilation depends on its quality and biological value.
When there’s no growth of young minks we detect nitrogen loss (endogenous and exchanging), besides the body uses it for energy (to compensate lack of carbohydrate and oil energy).
Thus, the connection between life weight growth and eaten protein lets us to count its ‘the life needed’ amount and growth.
Experimental data are presented in table 2, where x – a day growth, y – eaten digestive protein per head a day. During the extrapolation to the growth zero mark: the coefficient ‘a’ is a life needed protein (including exchanging and endogenous), ‘b’- g of protein for 1 g of growth.
Equation of the regression: For July $y = 0,421x + 22,4$; For September $y = 0,513x + 30,0$; For November $y = 0,499x + 25,2$.
In November minks had finished growth and moulting, it reflexes in lower digestive protein need for life support (per 1 kg exchange weight).
Then we used approximate dynamics of young minks life weight on the beginning and the end of month (Pereldik N.Sh. at al., 1987) to calculate the minks’ need in digestive protein according to the planned life weight to the 1st November. The example is in table 3.
Some growth of the need in September in comparison with the recommended by Pereldik N.Sh. at al. (1987) (on a 100 kKl of exchange energy 8 –9 g. of digestive protein) connected with the loss of biological value of dry protein for minks.
In September the growth of muscles stops but the moulting and winter fur forming is going on. It increases the need in qualitative protein.
In August and October the minks need may be defined with the methods of interpolation and previous and next months.
Thus, the norms of minks need in digestive protein are being proposed with the accounting of its’ life weight and the period of growing. The norms may be used as a base for mixed fodder ration calculation for minks.

Table 2 Calculation of the young minks need in digestive protein for life and growth.

Data	Growth period		
	July	September	November
N of minks	12	12	18
Average live weight (W), g	1032± 35	1476± 85	1907+ 45
Exchange weight, kg ($W^{0,73}$)	1,023	1,329	1,602
x, growth, g/animal/day	11,9± 5,4	-4,6± 2,8	4,6+ 2,2
y, eaten protein g/animal/day	27,5± 2,7	27,6± 2,8	28,1± 1,5
Coefficient ’в’	0,421	0,513	0,499
Coefficient ‘a’ g/animal/day	22,4	30,0	25,8
per 1 kg live weight	21,7	20,3	13,5
Per 1 kg exchanging energy ($W^{0,73}$)	21,9	22,6	16,1

Table 3 Calculation of need in digestive protein in young minks final live weight 2,3 on 1.11.

Data	Growth period					
	01.07	31.07	01.09	30.09	1.11	30.11
Planed live weight, kg (W)	0,81	1.35	1,80	2,21	2,30	2,23
Exchanging weight ($W^{0,73}$), kg	0,85	1.24	1,54	1,78	1,84	1,80
Average day growth, g	17,7		13,6		-2,3	
Life needed protein, g/animal/day	18,6	27,2	34,8	40,2	29,6	29,0
Protein for growth, g	7,45		6,98		-1,15	
General protein need, g/animal/day	26,1 — 34,7		41,8 — 47,2		28,8 — 27,9	
Per 100 kKl of exchange energy, g	9,1		10,9		7,7	
in % from exchanging energy	41,0		49,0		34,7	
According to Pereldik N.Sh. at al. (1987)						
Protein need, g per animal/day	27,6 — 31,1		37,2 — 41,9		28,4 — 32,0	

References:

Kulikov N.E. 2001. Metabolic nitrogen in minks fed with dry feed. Rebbirs and fur bearing animals breeding, 3, 12.

Kulikov N.E. 2002. Calculation method of metabolizable energy standards for young mink. Russian Agricultural Sciences, 3, 42-44.

Pereldik N. et al. 1987. Feeding of fur bearing animals. Agropromizdat, 342 p.

III – 13 P

Nourishing qualities of APK concentrate for minks' cubs

Elizaveta G.Kvartnikova, Andrey P.Kvartnikov
V.Afanasiev Research Institute of Fur Bearing Animals and Rabbits
Ramensky District, Moscow Region, Rodniki 140143, Russia

Abstract
The exchange of expensive protein in traditional feed composition by the protein of mixed feed, reduces substantially the feeding costs for mink. This work shows the results of a balance experiment on the use of nourishing substances APK concentrate, which was fed to minks kits of the colour type demi-buff. APK concentrate is a homogenous mix of crumbled feed of both animal and vegetable origin. Protein of APK consists of protein of animal origin at the rate of about 80%. Based on biochemical analysis, 100 g of APK concentrate contains, (g): dry substance - 90,2; organic substance - 85,3; raw protein - 27,3; raw fat - 2,5; carbohydrates - 55,5; including raw cellulose - 10,0; ashes - 4,5; gross energy - 1706 KJ. In the balance experiments coefficients of digestion of nourishing substances of APK concentrates in mink kits (%): dry substance - 66,0; organic substance - 67,0; protein - 68,4; fat - 70,1; carbohydrates - 62,4; energy - 85,8.APK concentrate contains per 100 g digestible nourishing substances (g): protein - 18,7; fat - 1,8; carbohydrates - 34,6; metabolic energy - 1016 KJ.

Introduction
Nowadays in Russian mink selection there are high expenses spent for feeding. They reach 70% from the cost of skin production.
One of the ways of reducing the price of animals' feedings is the replacement of a part of deficit and expensive animal protein in the diet with a protein of mixed fodder - concentrates.
In domestic fur farming it is recommend to use dry forages with up to 30 % of protein. It is mostly fish flour or unconventional forages. But a fish flour is quite an expensive product (G.S.Taranov, 1979), and unconventional forages have low nutritional value (N.E.Kulikov, 1999).
For reducing the price of minks' feedings and preservations of their high productivity there were developed a compounding and the technology of a mixed fodder - concentrate APK production.
The APK concentrate represents a homogeneous multicomponent mix of the crushed fodder of animal and vegetable origin, granulated and crushed. In structure of protein of APKconcentrate 80 % of a protein is of animal origin.
In the work the results of studying of nutritional value of APK concentrate for young growth of minksare represented.

Materials and Methods
Amino acid structure of APK concentrate was determined with the use of amino acid analyzer « Eppendorf-biotronics LC-3000 ».
Nutritional value for young growth of minks of APK concentrate was studied in balance experiment Kladovshikov, Samkov, 1975).
Experimental animals were put in the special exchange cages allowing separately to collect excrements and urine and providing absence of forages losses. The preliminary period for habituation took 3 days, registration - 4 days.
In experimental and control group there were 4 males of wild type brown mink in each.
On the tabulated data the daily diet of each animal contained 300 kcal
(1257 MJ) of exchange energy (for I control group - 260 g in crude weight; for II experimental - 180 g).
In the day of preparation of feed mixes there were taken their average tests for the definition of a chemical compound. In the beginning and the end of the experiment for all animals there was determined alive weight to 10g.
During the preliminary and registration periods minks daily at the same time received the defrozen portions of feed mixes. Excrements and urine were collected 3-4 times a day, preserved with 10 % of solution of hydrochloric acid (HCl). After the registration period there was defined weight of excrements and volume of urine, allocated by each animal. Up to the analysis urine was kept in a refrigerator, and average test of excrements were dried up to constant weight at 65°. The analysis of chemical compound of APK concentrate, feed mixes and minks' excrements were carried out in biochemical laboratory with the use of standard methods (Lukashik, Tashilin, 1965).

Results and discussion

According to the results of amino acids analysis, 100 g of APK concentrate contains, mg %: triptofan - 420; lizin-1950; histidin - 570; arginin-1280; treonin-1430; cistin-320; valin-1290; metionin-540; isoleycin-1230; leycin-1230; fenilalanin-1230.

The results of chemical compound of feed mixes APK concentrate are represented in table 1.

The results of balance experiment for each group of minks are represented in table 2.

According to the majority of investigated parameters there was marked statistically authentic difference between groups: at a zero gain of live weight in control group less excrements were allocated, in experimental group the maintenance of water in excrements is more upon identical allocation of water within urine, so the ways of allocation of water from the organism are redistributed: minks of II experimental group allocate the largest part of water (and waste products of a metabolism) with excrements, when in calculations on 100 g of dry substance of the consumed forage allocation of water in both groups was identical.

It indicates specific action of a APK concentrate on water balance which is more intense in group П.

By results of the analysis of excrement samples from each experimental mink there were calculated factors of the digestion of nutrients from diets (tab. 3).

Table 1 Chemical compound of provenders

Indexes	g/100g dry substance		
	1- control	II – experiment	APK concentrate
Dry substance	31,90	42,70	90,20
Organic substance	79,60	81,60	85,30
Grude protein	31,00	26,00	27,30
Grude fat	15,20	11,40	2,54
Carbohydrates	33,40	44,20	55,50
Including raw cellulose	1,62	6,57	9,97
Ash	13,81	11,14	4,5
Gross energy kcal|MJ	458,0/1919,0	437,7/1834,0	407,3/1706,6

Table 2 Water balance in experimental minks

Treatment	Groups		The degree of validity P <
	I-control	II-experiment	
Increase of live weight	0,0±1,0	-9,3±3,6	no
Food eaten, g/day	225,0	151,9	-
The amount of water in food	68,0	57,3	-
Excrements allocated, g/day	76,3±4,4	150,2±2,2	0,001
Dry substance in excrements, %	34,6±1,6	23,8±0,1	0,001
Water allocated, ml/day	120,6±3,7	173,7±10,5	0,01
Including within urine, ml/day	77,3±7,4	59,3±9,3	not
Including within excrements, ml/day	50,1±4,0	114,4±1,6	0,001
Water allocated on 100 g of dry substance, ml/day	180,8±5,6	168,8±10,7	no

The introduction of APK concentrate has seriously enlarged the digestion of dry substance and minerals, but brings down the digestion of organics, lipids, carbohydrates and energy. The digestion of protein practically has not changed. It is connected, probably, with its high (about 50 % from dry substance) inclusion in a diet.

The balance of nitrogen in minks was studied with the purpose of studying influence of APK concentrate on the animals' albuminous (tab. 4).

Minks of experimental group digested more nitrogen, than of control group. Experimental minks had authentically more nitrogen. Precipitation of nitrogen testifies to it in relative units (%), both to accepted, and to the digested nitrogen. The precipitation of nitrogen in percentage to digested characterizes quality of protein. It is called « seen biological value of a protein » (P. Mc. Donald at all. 1970).

True biological value of protein (IBC) was counted by formula:

$$IBC = \frac{(AKo + AMe + KPA + (PA - AK - AM}{PA - AK + AKo} \times 100\ \%$$

Where: AK - nitrogen of excrements; AM - nitrogen of urine, PA - precipitated nitrogen, AKo - exchange nitrogen of excrements, AMe - endohene nitrogen of urine, KPA - skin losses of nitrogen by hair, epithelium, perspiration (it is difficultly taken into account).

Biological value of protein of a experimental and control groups' diet is submitted in table 5.

The tested biological value of protein in a diet with APK concentrate appeared to be authentically above, than in a typical control diet. Such parameters as productive nitrogen, use of pure fiber and truly precipitated nitrogen also were higher. It testifies to the greater adequacy of protein of diet with APK concentrate to the requirements of young minks.

Knowing the rate of digestion of nutrients of the basic ration, the digestion of nutrients of the APK concentrate have been calculated with the use of differential method (tab. 6).

Thus, as a result of the work, there was established the nutritional value of APK concentrate which should be used at drawing up of diets of feeding for young.

Table 3 Digestion of nutrients from diets,%

Indexes	Groups		The degree of validity, P<
	I – control (main ration)	II-experiment (main ration+APK)	
Dry substance	60,8±0,8	65,3±0,6	0,01
Organic substance	71,0±0,9	67,7±0,7	0,05
Protein	64,6±1,7	67,8±1,7	not
Fat	94,0±0,3	89,7±0,7	0,01
Ash	7,2±2,5	53,6±2,6	0,001
Carbohydrates	66,0±0,8	61,8±0,4	0,01
Energy	74,3±0,9	70,8±0,8	0,05

Table 4 The balance of nitrogen in minks

Indexes	Groups		The degree of validity, P<
	I - control	II-experiment	
Eaten, g/day	3,31±0,01	4,29±0,03	0,001
Allokated with excrements, g/ day	1,17±0,06	1,38±0,07	not
Allocated with urine,g/ day	2,29±0,16	1,31±0,17	0,01
Digested,g/ day	2,14±0,06	2,91±0,09	0,001
Precipitated, g/ day	-0,15±0,19	1,6±0,20	0,001
Precipitated to eaten,%	-4,6±5,7	37,2±4,5	0,01
Precipitated to digested,%	-7,5±8,6	54,8±6,4	0,01

Table 5 Biological value of protein in minks

Indexes	Groups		The degree of validity, P<
	I - control	II-experiment	
The exchange nitrogen of excrements,g	0,17	0,26	-
The endohene nitrogen of urine,g	1,78±0,10	1,67±0,19	not
Productiv nitrogen,g	1,97±0,06	2,64±0,09	0,05
Realy digested nitrogen,g	2,31±0,06	3,17±0,09	0,001
Real biological value of protein,%	77,0±8,2	110,5±9,1	0,05
Truly digestability of protein,%	69,50±1,60	73,25±1,65	not
Use of pure protein,%	53,7±6,4	81,1±7,5	0,05
Use of nitrogen,%	2,67±0,32	3,38±0,31	not

Table 6 Digestabili of nourishing substances of APK cobcentrate

Treatment	Dry substance,g	Organic substance,g	Protein, g	Fat,g	Carbohyd-rates,g	Energy, Kcal/MJ
Factors of digestability, %	66,0	67,0	68,4	70,1	62,4	85,8
Presence digestible substances,%			18,7	1,78	34,6	242,6/1016,5

References:

Kladovshikov V.F., Samkov J.A. 1975. Studies of digestibletion of nourishing substances, balance of nitrogen and energy on fur animals. Methodic recommendation. 60 p.

Kulikov N.E. 1999. Foodcompozition as axcheng of protein in mixes of minks // Works of -breading: Diss….doctor of agricultural scinsis -362 p.VIJ, Dubrovisi. P. 181-183.

Mak-Donald P., Edvars R., Jrinchaly D. 1970. The nutriti on of animals. 502 p.

Taranov G.S. S1979. Scientific intodaction and practice use feshs meels and oserth dry foods in mink-

III – 14 RP

Individual *ad libitum* feeding of male + female pairs of mink kits during the growth period increases weight gain and feed efficiency

S.H. Møller, V.H. Nielsen**, B.K. Hansen***

** Department of Animal Health and Welfare and **Department of Animal Breeding and Genetics, Danish Institute of Agricultural Sciences, Research Centre Foulum, P.O. Box 50, 8830, Tjele, Denmark.*

E-mail steenh.moller@agrsci.dk

Abstract

Most farmed mink in Denmark are fed close to the average *ad libitum* intake during the growth period, based on feed leftovers at farm, shed or row level. Variation in voluntary feed intake between male + female pairs is ignored apart from the distribution of feed leftovers to cages without feed left over from the day before. Technological development has facilitated individual feeding and thus the possibility for true *ad libitum* feeding of mink. The variation in voluntary feed intake was studied in 174 male + female pairs of Scanbrown mink kits during 16 weeks from 11 weeks of age in July to 26 weeks of age in November. The feed allowance was adjusted Tuesday and Friday based on feed leftovers registered Monday + Tuesday and Thursday + Friday. The average feed intake was 44.3 kg per pair of kits equivalent to 395 g per day. The average weight gain was 2490 g per male + female pair. The average feed efficiency (g gain/kg feed) was 56 g/kg and in general, the feed efficiency increased with weight gain. The average difference between the lower and upper quartile of feed efficiency was 29% equal to an estimated difference in feed consumption of 15 kg for both quartiles to reach the average weight gain of 2.5 kg during the 16 weeks of growth. Compared to the normal feeding practice, individual *ad libitum* feeding provides the opportunity to utilise the full potential of the mink kits for growth and feed efficiency, and thereby for effective selection for these traits.

Introduction

Most farmed mink in Denmark are fed close to the average *ad libitum* intake during the growth period. The regulation of the daily feed allowance is based on feed leftovers from the day before at farm, shed or row level. Variation in voluntary feed intake between individual male + female pairs is to a large extent ignored apart from the distribution of feed leftovers to cages without feed left over. During the last decades mink farmers have selected for body weight in order to maximise their economic outcome. At the same time, the amount of feed and thus the main cost per pelt produced has increased accordingly, (e.g. from 35.5 kg in 1995 (Møller, 1998) + to 38.1 kg in 2003 (Møller, 2004)). Although the possibility to increase the feed efficiency has been documented (Berg & Lohi, 1992; Sørensen, 2002), the potential for reducing the feed cost per pelt produced has not been applicable in practice. Technological development of hand held computers (PDAs) has made individual feeding on each cage possible e.g. based on barcodes or other types of identification, and thus facilitated true *ad libitum* feeding of mink.

In a four-year experiment, the possibility of increasing the body size as well as the feed efficiency under different feeding strategies is investigated. Three lines of male+female pairs are fed either according to normal farm practice, restricted (10% below farm practice) or *ad libitum*. The present paper describes the results in terms of weight gain, voluntary feed intake and feed leftovers of the first growth season, with special emphasis on the *ad libitum* fed line.

Material and Methods

Animals: The mink in the present feeding experiment are the base population in a selection experiment established by crossing two Scanbrown lines previously selected for high November weight, and for high weight as well as litter size (Nielsen et al., 2004). Three selection lines with different feeding strategies: Farm Feeding (FF), *Ad Libitum* (AL) and Restricted Feeding (RF) were established in December 2002 each with 100 females. The feeding strategies were applied to 198, 192 and 192 male + female pairs of kits from line FF, AL and RF, respectively, in the period from weaning in July to live animal grading in November.

Feeding: The animals were separated in male + female pairs and weighed within three days in late June and the test feeding was commenced in early July on the same day for all three lines. The mink were fed a commercial standard diet delivered daily from the local feed kitchen. The feed allowance was regulated by feed leftovers registered at 9 a.m. 2 hours before the daily feeding. The leftovers were graded as no feed left, Grade 1: less than 5 square inches left (what will be eaten before next feeding) and Grade 2: more than 5 square inches left. Leftovers were registered Monday + Tuesday and Thursday + Friday and regulated Tuesday and Friday. The control line FF was fed the same amount on each cage and feed leftovers were fed to cages with no feed left, according to normal Danish farm practice. Occasional excessive feed leftovers were collected and weighed. The feed allowance to line FF was regulated in the following manner:

- Grade 2 leftovers on less than 33% of the cages both days: The allowance was increased by 20 g per cage per day.
- Grade 2 leftovers on more than 66% of the cages both days: The allowance was reduced by 20 g per cage per day.

Line RF was kept under a restrictive feeding regime and fed 90% of the amount of feed offered to the control line FF. Occasional feed leftovers were collected and weighed.

Line AL was fed *ad libitum* at cage level and feed leftovers were collected before feeding and weighed each day. The feed allowance was regulated in the following manner:

- No leftovers one or both days: The allowance was increased by 20 g per cage per day.
- Grade 2 leftovers both days: The allowance was reduced by 20 g per cage per day.

An overview of the regulation of the feed allowance in each line is given in Table 1.

The feed allowance at cage level in all three lines was controlled by a computerised feeding machine regulated by a Palm Pilot (the Farm Pilot used in the breeding programme from Copenhagen Fur Center). By reading a bar code on each cage the Individual Feeding programme developed by “Tved Maskinbyg” pumped out the pre-programmed daily ration on each cage.

Data: The kits were weighed individually every three weeks from separation to live animal grading in November. Eight weights were recorded for each animal. The feed allowance was recorded as the amount of feed registered by the Farm Pilot. The Farm Pilot feeding was measured to give on average 8.4 g or 2.2% more feed than programmed. If programmed to feed 380 g the feed allowance was measured to 388.4±26.9 g. However, if the actual amount of feed was more than 20 g above or below the pre-programmed amount, the actual amount was registered by the Farm Pilot and used in the calculations.

Feed leftovers on each cage in each line were observed most weeks on Monday, Tuesday, Thursday and Friday and a total of 82 observation days were obtained. The total feed consumption for each cage was calculated as the total feed allowance in the period minus the weight of collected feed leftovers in the period attributed to each cage relative to the number of feed leftovers observed. For the feeding experiment, recordings from 16 weeks (week 30 to 45, 11 to 26 weeks post partum) were used for data analysis from 184, 174 and 178 pairs of kits in line FF, AL and RF, respectively.

Statistics: The average weekly feed consumption in each line was calculated as the average feed allowance minus the weekly average of leftovers per cage (male + female pairs of kits). The average weight gain and standard deviation from 11 to 26 weeks of age was calculated for male + female pairs of kits in each line. The feed efficiency was calculated as the weight gain in g divided by the feed consumption in kg for each pair of kits. The average feed efficiency and standard deviation from 11 to 26 weeks of age was calculated for each line.

Table 1. Feed regulation of male + female pairs of kits from 11 to 26 weeks of age in line FF on farm feeding, line AL on *ad libitum* feeding and line RF on restricted feeding.

Line	N	Feed leftovers registered	Observation	Feed allowance
AL	174	Mon+Tue and Thu+Fri	no leftover one or both days > 5 ” leftover both days	increased 20 g decreased 20 g
FF	184	Mon+Tue and Thu+Fri	> 5 ” leftover on < 33% of the cages > 5 ” leftover on > 66% of the cages	increased 20 g decreased 20 g
RF	178		none	90% of line FF

Results and Discussion

The three different strategies for regulating the feed allowance succeeded as planned in the sense that the average feed allowance to line AL and RF was 10 % above and below the feed allowance for line FF, respectively, almost every week from 8 to 29 weeks post partum. On average 4.7 kg of feed leftovers were collected per cage equal to 42 g daily in line AL while excess of feed very rarely had to be collected from line FF or RF. When these feed leftovers were subtracted, the weekly feed consumption in line AL was only a few % higher than in line FF (Fig 1). The kits in the three lines responded to the different feeding regimes by significant differences in weight gain (Fig 2).

Figure 1. Average daily feed consumption by male+female pairs of kits from 8 to 29 week of age in line FF on farm feeding, line AL on *ad libitum* feeding and line RF on restricted feeding

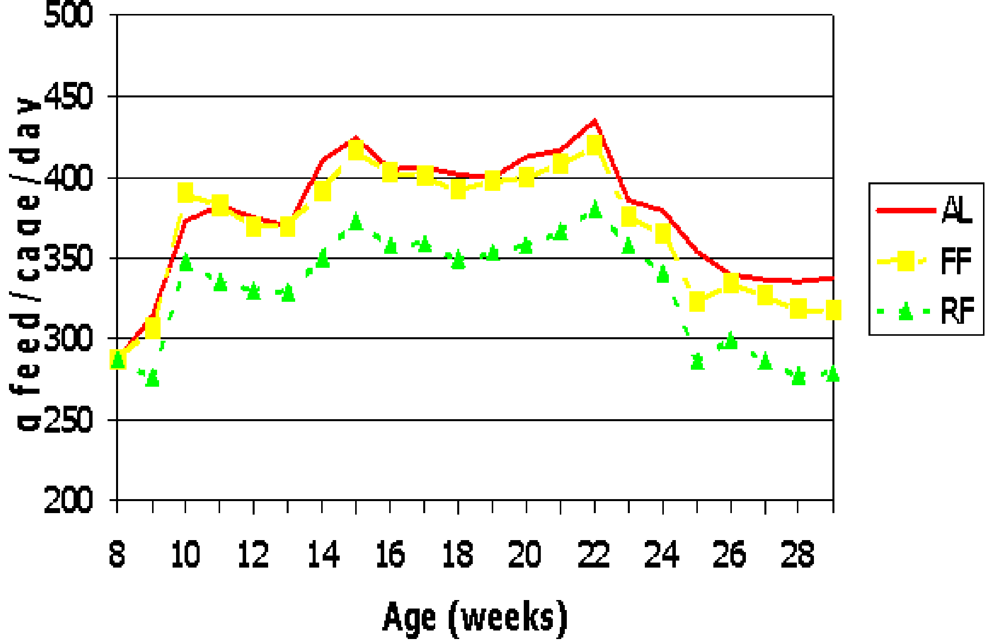
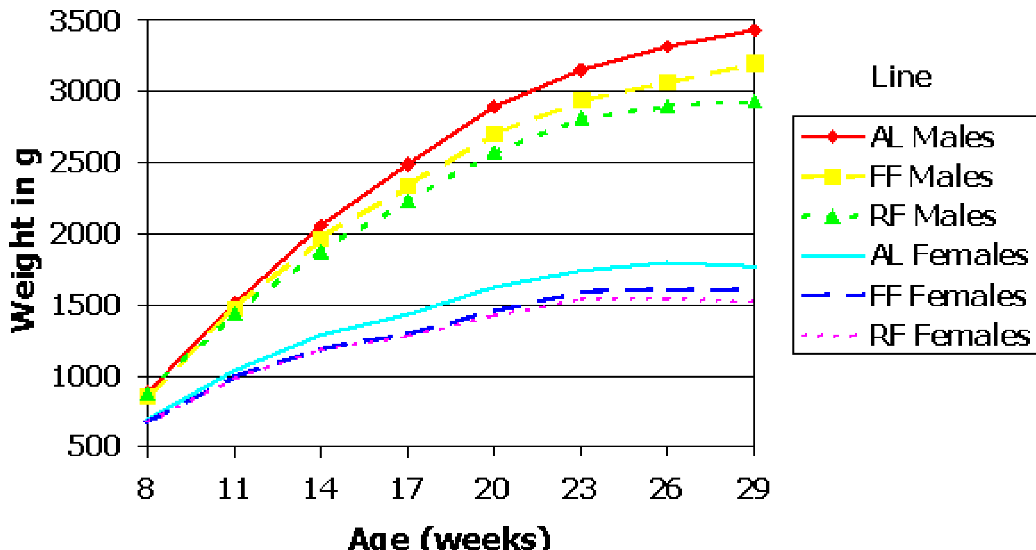


Figure 2. Weight development of kits from 8 to 29 weeks of age in line FF on farm feeding, line AL on *ad libitum* feeding and line RF on restricted feeding



The average feed consumption in all lines from 11 to 26 weeks post partum was 42.06 kg per male+female pair of kits. This resulted in a weight gain of 2239 g and thus an average feed efficiency of 53.13 g of gain per kg feed consumed. The individual *ad libitum* feeding resulted in the highest weight gain, feed consumption and feed efficiency. The restricted feed allowance in line RF reduced the weight gain but at the same time increased the feed efficiency compared to line FF (Table 2).
The application of individual ad libitum feeding in line AL increased the weight gain by 276 g (or 12%) by use of only 1.1 kg (or 2.6%) of extra feed, compared to line FF resulting in a very high average efficiency of 244 g of gain per kg extra feed consumed, due to the individual *ad libitum* feeding. An explanation for this can be that the more appropriate distribution of almost the same amount of feed gave the mink in the AL line the opportunity to utilize their potential for growth better than the mink in the FF line. This further implies that under Farm Feeding practice, pairs of kits with low appetite are fed *ad libitum,* pairs of kits with average appetite are fed close to *ad libitum* while pairs of kits with large appetite are fed a restricted amount of feed, and the distribution of feed leftovers does not compensate the pairs of kits with large appetite.
The feed efficiency ranged from 24 to 83 g gain/kg feed for individual male+female pairs of kits and in all three lines there was a substantial variation in feed efficiency, as the coefficient of variation was 18% in line FF, 13% in line AL and 13% in line RF. The larger variation in line FF may be due to how the farm feeding is experienced by the mink, depending on their appetite as suggested above. The smaller variation in line AL and RF may then be explained by the fact that all the mink in these lines experience either *ad libitum* or restricted feeding, respectively. This explanation is supported by the fact that grade 2) leftovers were observed on average 31 ± 15, 37 ± 5 and 6 ± 7 times in line FF, AL and RF, respectively, out of 82 observation days. In line FF and RF the same cages had grade 2) leftovers most days while grade 2) leftovers were more randomly distributed among cages in line AL, due to the strategy for regulation of the feed allowance.
In the *ad libitum* fed line the average feed efficiency in the best 25% of the line was 65.56 g gain/kg feed consumed compared to 47.38 in the lowest 25%. This difference of 28% in feed efficiency between the upper and lower quartile was equal to an estimated difference in feed consumption of 14.6 kg (38.0 kg in the upper quartile and 52.6 kg in the lower quartile) if the mink pairs from each quartile should reach the average weight gain of 2490 g in the *ad libitum* fed line. The average weight gain and feed consumption in the upper and lower feed efficiency quartiles, as well as in the middle 50% of line AL is given in Table 3.
The feed efficiency in line AL was highly correlated to weight gain (r=0.91) and moderately correlated to feed consumption (r=0.37). The high correlation between feed efficiency and gain confirms the results by Berg & Lohi (1992) measured at group level and by Sørensen (2002) measured over four weeks in July.

Table 2. Average weight gain, feed consumption and feed efficiency of male + female pairs of kits from 11 to 26 weeks post partum in / of age in line FF on farm feeding, line AL on *ad libitum* feeding and line RF on restricted feeding.

Line	N	Weight gain, g	Feed consumption, kg	Feed efficiency, g/kg
AL	174	2490 ± 438	44.26 ± 3.50	56.05 ± 7.3
FF	184	2214 ± 411	43.13 ± 0.22	51.31 ± 9.4
RF	178	2020 ± 253	38.76 ± 0.56	52.13 ± 6.6

Table 3. Average weight gain, feed consumption and feed efficiency of male + female pairs of kits from 11 to 26 weeks of age in the upper and lower feed efficiency quartiles as well as in the median 50 % of line AL on *ad libitum* feeding.

Quartile of line AL	N	Weight gain, g	Feed consumption, kg	Feed efficiency, g/kg
75%	43	3008 ± 350	45.82 ± 3.44	65.56 ± 4.6
50% Median	87	2464 ± 246	44.17 ± 3.39	55.74 ± 2.7
25%	44	2035 ± 222	42.91 ± 3.23	47.38 ± 3.3

However both authors found a negative correlation between feed consumption and feed efficiency. The large variation in feed consumption in line AL that is only moderately correlated to feed efficiency indicates that it should be possible to increase the feed efficiency without increasing the feed consumption. A relatively high heritability for feed efficiency in July of 0.30 has been calculated by Sørensen (2002). If the same is true for the entire growth period feed efficiency can be rapidly improved by selection, when the practical management tools for registration of the feed consumption and controlling the feed allowance are available.

The large variation in weight gain has for many years been exploited in selection programmes while selection for feed efficiency has not been performed due to lack of management and feeding technology. Despite the positive genetic correlation between weight gain and feed efficiency (Sørensen, 2002), selection for weight under farm feeding conditions may not be an effective way to improve feed efficiency due to different feeding regimes experienced by the mink. Individual ad libitum feeding during the growth period provides a homogenous environment allowing the full expression of the growth potential, and may thereby facilitate improvement of feed efficiency in mink production.

Literature

Berg, P. & Lohi, O., 1992. Feed consumption and efficiency in paternal progeny groups in mink. Acta Agric. Scand. Sect. A., Animal Sci. 42, 27-33.

Møller, S.H. 1998. N input and output on a mink farm. Mink, Appendix 1 in Poulsen, H.D. & Kristensen V.F. (eds.) Standard Values for Farm Manure. DIAS report No. 7 Animal Husbandry. 146-149.

Møller, S.H. 2004. N input and output on a mink farm. Mink, in Poulsen, H.D. & Kristensen V.F. (eds.) Standard Values for Farm Manure. DIAS report No. 7 Animal Husbandry. (In press).

Nielsen, V.H., Møller, S.H., Hansen, B.K. and Berg, P. 2004. The effect of *ad libitum* and restricted feeding on growth curves in mink selection lines. Scientifur 28. Proceedings of the ??th International congress in fur animal production. September 15-18th The Netherlands.

Sørensen, K. 2002. Selection for feed efficiency in mink (Mustela vison). Ph.D. Thesis. 108 pp.

III – 15 RP

Effects of dietary methyl donors on health status in blue fox (*Alopex lagopus*) vixens given a low protein diet during body fat mobilisation

Nita Nenonen[1], Petteri Nieminen[2], Tuula Dahlman[3], Jarmo Valaja[4], Ilpo Pölönen[3], Marjukka Anttila[5], Anne-Mari Mustonen[2] and Teppo Rekilä[1]

[1] *MTT, Agrifood Research Finland, Animal Production Research, Fur Animals, FIN-69100 Kannus, Finland*

[2] *University of Joensuu, Department of Biology, P.O.Box 111, FIN-80101 Finland*

[3] *Finnish Fur Breeders Association, P.O.Box 5, FIN-01601 Vantaa, Finland*

[4] *MTT, Agrifood Research Finland, Animal Production Research, Animal Nutrition, FIN-31600 Jokioinen, Finland*

[5] *National Veterinary and Food Research Institute, EELA, P.O.Box 45, FIN-00581 Helsinki, Finland*

Abstract

The feeding trial was carried out on 60 blue fox vixens before the breeding season (age six months at the beginning of the trial). The aim of the present study was to find out to which extent low dietary protein supplemented with methyl donors affects fat and liver metabolism and body weight reduction in the period November-March. Treatments were: control (blue fox feed, 20% protein from ME), Alimet® (control feed with supplemental methionine hydroxy analogue), betaine (control feed with supplemental betaine), choline (control feed with supplemental choline), methionine (control feed with supplemental DL-methionine) and positive control (commercial blue fox feed, 35 % protein from ME). Body weight loss, blood parameters, liver histology, enzyme activities, and cortisol:creatinine ratio of urine were measured. In conclusion, the experiment showed that methyl donors had no effect on health status on blue fox vixens given a low protein diet during the body fat mobilisation period. Generally, the low dietary protein level was reflected in some health parameters, but no animals revealed symptoms of malnutrition. The results demonstrated that the blue fox have extreme abilities to maintain health on a suboptimal diet during body fat mobilisation.

Introduction

Farmed blue foxes are typically very obese before the onset of the breeding season. First year breeders often have higher body weights than older females. In practice, high body weights have been shown to be negative for the reproduction result in blue fox. Therefore they must reduce body weight during November to March to be prepared for the mating season. This probably causes stress to animals and affects on liver metabolism. In the liver many metabolic reactions take place, such as detoxifying functions, secretion of bile etc. Animals in negative energy balance may have fatty liver (*hepatic lipidosis*). In chronic cases chirrosis of the liver can ensue. Thus, blue fox vixens for breeding should be selected earlier in the autumn than at present to avoid excessive obesity during the breeding season.

Choline, betaine and methionine have different functions in the metabolism, except they can all act as methyl donors, needed in fat metabolism and to prevent fatty liver. While choline and betaine are classified as vitamins/precursors, methionine is an amino acid. Fatty liver is common finding in fur animals at post mortem investigations. Fatty liver may be the primary or secondary cause of death. Fatty liver appears mostly in the spring among blue fox vixens.

The aims of the present study were: to find out to which extent low protein level in feed supplemented with methyl donors affect fat and liver metabolism during rapid dieting and weight declining period in winter.

Material and Methods

Experimental design and management

The experiment was carried out at the fur animal research station of Kannus, MTT Agrifood Research Finland. The experiment was conducted with 60 blue fox vixens. Vixens were six months old and average body weight was 13.4 kg (±0.9 kg SD) at the beginning of the trial. It lasted for 120 days, from November 11th 2002 to March 10th 2003. For the first four weeks the animals were fed with high-energy commercial blue fox feed (8.0 MJ/kg feed).

After December 14th (average body weight 14.5 kg ± 0.1 SD) the animals were fed with experimental diets to cause weight loss until the end of the trial (energy allowance 1.14 MJ day^{-1}). The animals were maintained according to common farming practices in wire mesh cages, one animal per cage. In the trial experimental animals were blocked according to litter, and one full-sib pairs were assigned to separate experimental groups (6 x 10 animals).

Treatments:

1. Control: blue fox feed, 20% protein from ME
2. Alimet®: control feed with methionine hydroxy analogue
3. Betaine: control feed with supplemental betaine
4. Choline: control feed with supplemental choline
5. Methionine: control feed with supplemental DL-methionine
6. Positive control: commercial blue fox feed, 35 % protein from ME

After December 14th the diets 1-5 contained Baltic herring, chicken slaughterhouse by-products, cooked and dehydrated barley, rape seed oil, molasses, oat hull meal and FPF-vitamin mix without methionine. Protein from ME was sub optimal in feeds 1-5 in order to maximize the use of proteins and methyl groups. The diets were identical and isocaloric, except for the methyl donor. Betaine, methionine, choline and methionine hydroxy analogue were added to yield the number of methyl groups in positive control. The positive control was formulated on the basis of the recommendation of the Finnish Fur Breeders' Association with added methionine (total methionine in feed 8 g/kg DM) . The experimental diets were produced in one batch and stored frozen until use. Compositions and chemical analyses of the diets are shown in Tables 1 and 2.

Recordings, chemical and statistical analyses

The animals had free access to water and were reared singly outdoors in conventional pelter cages in a two-row shed. The animals were weighed every second week during the experiment. Food consumption was measured daily. At the end of the trial blood samples were collected before the euthanasia. Blood ALT, AST, creatinine kinase, glucose, HCT, HGB, chol, total prot, RBC, triglyserides, WBG, LDL, HDL, uric acid,

Table 1. Composition of the diets 1-5 (Control, Alimet®, Betaine, Choline, Methionine) and Positive Control

Ingredients, %	Diets 1-5	Positive Control
Baltic herring	10.2	25.0
Chicken slaughterhouse offal	17.4	20.0
Barley, cooked dehydrated	20.5	7.0
Protein mix		8.0
Oat hull meal	3.1	3.0
Molasses	2.0	2.0
Rape seed oil	2.0	0.5
FPF-vitamin mix	1.5	1.5
Potassium sorbate	0.03	0.03
Vitaquine	0.1	0.1
Water	43.0	32.9

Table 2. Chemical analyses of the diets 1-5 (Control, Alimet®, Betaine, Choline, Methionine) and Positive Control

	Diets 1-5	Positive Control
Dry matter (DM), g/kg	350	355
Ash, g/kg	17	22
Crude protein, g/kg	67	121
Fat, g/kg	62	65
Carbohydrate, g/kg	204	146
MJ kg DM	15.8	16.7
Metabolisable energy (ME):		
Protein, %	20.6	34.7
Fat, %	40.4	39.3
Carbohydrate, %	39.0	26.0

ammonia, glycerol, bilirubin and creatine were analysed.

Animals were slaughtered by electrocution. The liver and adrenal glands were weighed. Liver dry matter and fat% were analysed and hepatocyte vacuolisation was evaluated. The enzyme activities of liver and kidney were determined

spectrophotometrically. Urine cortisol and creatinine were analysed at the beginning and in the end of the experimental period and the cortisol:creatinine ratio was calculated. The data was statistically analysed by the GLM procedure of SAS.

Results

Average body weight loss was approximately 29% during period November-March. Food consumption and body weight loss were not different between the experimental groups at any weight recording (Figure 1). Liver weight, liver dry matter and liver fat% were not different between groups (Table 3). Hepatocyte vacuolization was significantly lower in the positive control group compared to all the other groups (Table 3).

Blood parameters were not significantly different between groups except ALT, chol and prot (Table 4). ALT and cholesterol were significantly lower in the positive control group in comparison to the choline group. ALT was higher in choline group compared to betaine group. Plasma total protein was significantly higher in the positive control group compared to the betaine and control group.

Figure 1. Weight gain of the blue fox vixens

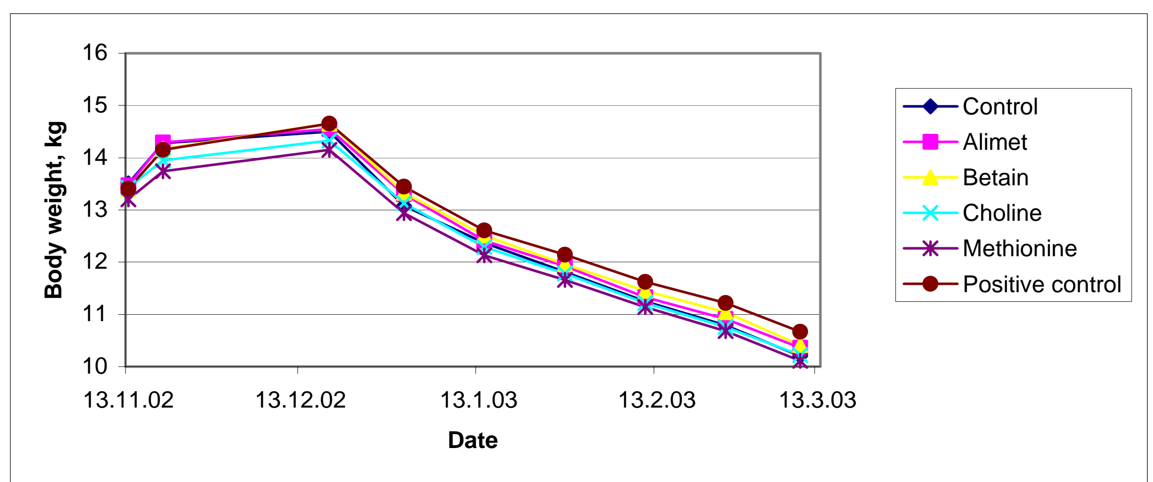


Table 3. Liver weight, liver fat-%, liver dry matter, fat% in dry matter and hepatocyte vacuolisation by arbitrary scale of 0-4 (0=nil, 1=very mild, 2=mild, 3=moderate, 4=moderately severe), (mean±SE).

	Control	Alimet®	Betaine	Choline	Methionine	Positive control	P*
Liver, g	171.0 ± 11.9	161.0 ± 5.7	170.6 ± 7.0	170.4 ± 11.8	163.5 ± 7.3	150.6 ± 4.7	NS
Liver fat %	6.2 ± 0.9	5.1 ± 0.3	6.6 ± 1.0	6.3 ± 0.9	5.1 ± 0.6	3.9 ± 0.2	NS
Liver DM	32.0 ± 0.7	31.5 ± 0.4	32.4 ± 0.6	31.9 ± 0.7	31.4 ± 0.4	31.5 ± 0.3	NS
Fat % in DM	18.9 ± 2.4	16.2 ± 0.9	19.8 ± 2.6	19.2 ± 2.4	16.2 ± 1.5	12.5 ± 0.6	NS
Hepatocyte vacuolisation	2.50 ± 0.4^{b}	2.20 ± 0.2^{b}	2.22 ± 0.3^{b}	2.10 ± 0.4^{b}	2.20 ± 0.3^{b}	0.30 ± 0.2^{a}	<0.001

** NS = not significant, P < 0.05 = significant difference between groups*

Table 4. Blood parameters of the blue fox vixens (mean ±SE)

	Control	Alimet®	Betaine	Choline	Methionine	Positive control	P*
ALT U/l	113.9^{ab} ± 22	128.5^{ab} ± 14	103.6^{a} ± 18	178.9^{bc} ± 18	141.2^{ab} ± 19	97.3^{a} ± 14	<0.05
AST U/l	31.8 ± 1.1	26.5 ± 2.2	26.0 ± 1.3	33.4 ± 4.2	29.00 ± 1.8	27.3 ± 2.1	NS
Creatine kinase U/l	150.3 ± 28	133.0 ± 16	79.7 ± 5.0	129.3 ± 29	122.0 ±10	103.8 ± 9	NS
Glucose mmol/l	6.90 ± 0.2	7.65 ± 0.4	7.16 ± 0.5	6.77 ± 0.3	6.79 ± 0.3	7.29 ± 0.6	NS
HCT %	51.9 ± 1.1	53.3 ± 0.9	52.1 ± 1.2	54.8 ± 1.0	52.5 ± 1.0	52.1 ± 1.3	NS
HGB g/l	163.3 ± 0.3	165.1 ± 0.2	162.1 ± 0.2	168.8 ± 0.2	164 ± 0.3	168.7 ± 0.2	NS
Chol mmol/l	5.54^{ab} ± 0.2	5.65^{ab} ± 0.1	5.64^{ab} ± 0.3	5.84^{b} ± 0.2	5.69^{ab}± 0.2	4.87^{a} ± 0.3	<0.05
Total prot g/l	54.7^{a} ± 0.8	58.6^{ab} ± 1.0	56.4^{a} ±0.8	58.0^{ab} ± 1.0	57.4^{ab} ± 0.6	60.6^{b} ± 1.3	<0.05
RBC10^{12}/l	8.97 ± 0.1	9.02 ± 0.1	9.01 ± 0.1	9.09 ± 0.1	9.01 ± 0.1	8.67 ± 0.3	NS
TG mmol/l (triglycerides)	0.69 ± 0.1	0.70 ± 0.1	0.38 ± 0.0	0.72 ± 0.1	0.59 ± 0.0	0.73 ± 0.1	NS
WBC 10^{9}/l	6.34 ± 0.4	5.91 ± 0.6	6.90 ± 0.9	6.99 ± 0.8	7.88 ± 0.8	6.69 ± 0.5	NS
LDL mmol/l	0.24 ± 0.0	0.23 ± 0.0	0.32 ± 0.1	0.25 ± 0.0	0.24 ± 0.0	0.23 ± 0.0	NS
HDL mmol/l	4.76 ± 0.3	4.53 ± 0.3	4.86 ± 0.3	4.67 ± 0.2	4.97 ± 0.3	4.16 ± 0.3	NS
Uric acid µmol/l	23.6 ± 4.3	28.0 ± 4.1	25.5 ± 3.5	32.2 ± 4.4	18.9 ± 2.9	32.4 ± 5.3	NS
Ammonia µmol/l	229.7 ± 4.9	233.5 ± 4.9	236.3 ± 7.3	221.6 ± 12.5	231.3 ± 9.4	227.0 ± 7.1	NS
Glycerol µmol/l	484.2 ± 60	476.4 ± 51	431.3 ± 40	471.0 ± 40	443.3 ± 43	584.0 ± 44	NS
Bilirubin µmol/l	4.01 ± 0.3	4.93 ± 0.4	4.14 ± 0.4	5.33 ± 0.4	4.27 ± 0.4	4.66 ± 0.6	NS
Creatinine µmol/l	85.7 ± 7.6	77.8 ± 6.7	84.0 ± 7.4	89.2 ± 8.6	67.1 ± 8.6	82.4 ± 7.2	NS

** NS = not significant, P < 0.05 = significant difference between groups*

Liver glycogen content was significantly lower in the positive control group in comparison to the choline group (Table 5). Kidney lipase activity was significantly higher in the positive control than in the Alimet®, betaine or methionine groups. Liver glucose-6-phosphatase activity was higher in the positive control group than in the Alimet®, betaine, choline and methionine groups. Kidney glycogen, liver and kidney phosphorylase, liver and kidney lipase and kidney glucose-6-phosphatase activities were equal in all groups. Weights of adrenal glands and cortisol:creatinine ratio in all groups was equal.

Table 5. Enzyme activities of the livers and kidneys of the blue fox vixens (mean ±SE)

	Control	Alimet®	Betaine	Choline	Methionine	Positive control	P*
Liver glycogen mg/g	32.8 ± 3.9[ab]	33.7 ± 4.6[ab]	30.1 ± 4.4[ab]	37.2 ± 5.9[a]	31.2 ± 4.0[ab]	17.3 ± 3.1[b]	<0.05
Kidney glycogen mg/g	1.43 ± 0.14	1.87 ± 0.38	1.28 ± 0.15	1.64 ± 0.33	1.43 ± 0.20	2.17 ± 0.61	NS
Liver phosphorylase µg P/mg/h	54.1 ± 4.2	49.1 ± 3.8	47.6 ± 4.0	47.1 ± 3.2	48.0 ± 4.4	48.4 ± 4.0	NS
Kidney phosphorylase µg P/mg/h	3.73 ± 0.18	2.80 ± 0.15	3.58 ± 0.80	3.18 ± 0.25	3.79 ± 0.38	3.70 ± 0.20	NS
Liver lipase µg 2-naphthol/mg/h	25.6 ± 2.1	23.4 ± 2.4	24.6 ± 2.5	29.6 ± 3.0	23.1 ± 2.8	26.6 ± 2.2	NS
Kidney lipase µg 2-naphthol/mg/h	27.9 ± 2.5[ab]	19.5± 1.6[a]	20.0 ± 1.5[a]	23.7 ± 3.4[ab]	16.9 ± 1.8[a]	34.8 ± 6.0[b]	<0.05
Liver glucose-6-phosphatase µg P/mg/h	16.6 ± 2.3[ab]	15.3 ± 1.0[a]	14.7 ± 2.1[a]	14.7± 1.2[a]	15.6± 2.6[a]	24.1 ± 2.2[b]	<0.05
Kidney glucose-6-phosphatase µg P/mg/h	9.23 ± 1.38	7.77 ± 0.49	7.91 ± 0.54	8.75 ± 0.54	7.87 ± 0.66	8.09 ± 0.44	NS

** NS = not significant, P < 0.05 = significant difference between groups*

Discussion

Weight loss was similar in all groups as it was expected because of the same average energy consumption in the groups. All parameters except ALT were not different in the control, Alimet®, betaine, choline and methionine groups. These supplementation levels of methyl donors with sub optimal protein level did not affect liver metabolism with these weight loss levels. The positive control group seemed to be the healthiest in most measured parameters. There were no signs of fatty liver in the positive control group and only few indications in the other groups. Probably the amino acid content was better in the positive control group and, as a consequence, their plasma ALT and AST activities were at the lowest observed level indicating a very low probability of any liver damage due to weight loss. The diet of the control group contained 35 % protein from ME and the other diets approximately 20% protein from ME.

Normally the liver contains 2-4 % fat of the total weight. In studies of Juokslahti et al. (1978) fatty livers contained more than 50 % fat of total weight. Mink liver contains more fat than liver from blue fox (Ahlstrøm & Skrede, 1997). In Sweden mink livers contained 4.3-6.7 % fat at pelting (Alden et al. 1997). In our study liver fat contents of the blue fox vixens were 3.9-6.6 %, indicating quite good state of liver. However the positive control groups' liver hepatocyte vacuolisation was almost nonexistent in contrast to mild or moderate changes in liver hepatocytes of other groups.

The blood parameters did not reveal that the animals suffered from severe malnutrition from the low protein level. The activities of liver and kidney enzymes taking part in the carbohydrate or lipid metabolism were almost at the same level in animals receiving low protein as for those in the positive control. Thus blood glucose and lipid levels were maintained almost at the same level at the different feeding regimes.

Weight loss didn't affect the general well being of the blue fox vixens before the breeding season. Cortisol:creatinine ratios of the urine and the adrenal gland mass were similar in all groups. Long-term stress is thought to cause an increase in adrenal gland mass weight (Selye, H. 1950). The urine cortisol:creatinine ratio indicates stress level and is assumed to be more reliable way to measure adrenal function than straightforward blood cortisol values (Beerda, M. et al. 1996). Urinary cortisol was expressed as the cortsol:creatinine ratio to correct for variation in the dilution of urine (Lasley, B.L. et al. 1991, Novak et al. 1989).

The experiment ended just before the mating season started. It is possible that the experimental feeding of the low protein diets could have given more pronounced effects during coming mating season even if the dietary protein level had been increased.

The positive control feed (35% protein of ME) was formulated on the basis of current recommendation (38% protein of ME) from the Finnish Fur Breeders' Association. Animals maintained good health in the positive control group. Therefore the recommended feed and a weight loss of approximately 29 % in

three months could be safely introduced. Optimum protein level could be lower than recommended 38% protein of ME.

In conclusion, the experiment showed that methyl donors had no effect on health status on blue fox vixens given a low protein diet during the body fat mobilisation period (November-March). Generally, the low dietary protein level was reflected in some health parameters, but no animals revealed symptoms of malnutrition. The results demonstrated that the blue fox have extreme abilities to maintain health on a suboptimal diet during body fat mobilisation.

Acknowledgements

This research was funded by The Helve Foundation.

References

Ahlstrøm and Skrede. 1997. Liver fatty acid composition and peroxisomal fatty acid activity in blue foxes (*Alopex lagopus*) and mink (*Mustela vison*) fed diets containing different levels of fish oil. Comp. Biochem. Physiol. Vol 117A, No.1 pp.135-140.

Alden, E., Persson, U., Järplid, B. and Mejerland, T. 1998. Analysis av ALAT-innehåll hos svensk farmuppfödd mink 1997. Våra Pälsdjur 69:3, 52-56

Beerda, M., Schilder, M.B.H.,Janssen, N.S.C.R.M., Mol, J.A. 1996. The use of saliva cortisol, urinary cortisol, and catecholamine measurements for a noninvasive assessment of stress responses in dogs. Horm. Behav. 30:272-279

Juokslahti, T., Lindberg, P., Pekkanen, T. and Sjögård, B. 1978. Cholinechloride in the treatment of fatty liver in mink. Scientifur, 2:4, 35-38.

Lasley, B.L., Kirkpatrick, J.F. 1991. Monitoring ovarian function in captive and free-ranging wildlife by means on urinary and fecal steroids. J.Zoo Wildl. Med. 22:23-31,

Novak, M.A., Drewsen, K. H. 1989. Enriching the lives of captive primates. In: Segal, E.F., Ed. Housing, care and physiological wellbeing of captive and laboratory primates. Park Ridge, NJ: Noyes Publications, pp. 162-164.

Selye,H. 1950. The physiology and pathology of exposure to stress. Montreal : Acta, Inc.

III – 16 RP

Some biochemical parameters in serum of mink fed high energy feedstuff with antioxidant and preservative supplement*

Hanna Bis-Wencel, Leon Saba, Antoni Kopczewski[1], Bożena Nowakowicz-Dębek, Wioletta Wnuk
Laboratory of Reproduction Biology of The Department of Animal and Environment Hygiene, The Faculty of Biology and Animal Breeding,UA in Lublin, ul.Akademicka 13,20-9510 Lublin, E-mail: hanka13@poczta.onet.pl , POLAND.
[1]The Subdepartment of Veterinary Hygiene, ul.Kaprów 10,80-316 Gdańsk, POLAND.

* This work was conducted as part of the research project No. PO6Z 0512 25 financed by the State Committe for Scientific Research (2003-05)

Abstract
The objective of the investigations was to determine a variability range of reference values of the chosen biochemical parameters in the serum of minks at varied levels of energy feeding. At the some time the antioxidant and preservative supplement at different rate was used. The studies were performed at the mink farm „C" situated in Poland. Blood was collected from heart puncture from mink yearlings twice in December. In serum of animals the enzyme activity of aspartic aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), a level of urea, creatinine, glucose, bilirubin, and uric acid were determined by the spectrophotometric method with monotests of Cormay. The obtained results showed substantial activity increase of ALT, AST in D_2 group and LDH in both experimental groups. The other parameters were found within the ranges presented by other authors.

Introduction
The widespread introduction of new feeding methods of carnivorous fur animals by the leading countries needs complex assessment of their impact on animal health and performance. The new feeding methods consist in an increase of dietary energy through increased content of energy from fat, that as a consequence induces higher reproduction indices as well as skin of the optimum quality parameters. It may however, lead to enhanced disturbances of metabolism at the cellular level, unnoticeable over the short animal life. This fact has been mentioned in few scientific papers and breeders` observations (Sławoń,1986 and Winnicka,1997). The feedstuff including fish offal, poultry wastes and slaughterhouse offal requires the use of preservatives and antioxidants that are not indifferent for animal health. The present authors` earlier investigations indicate the pathological changes in the intrinsic organs of polar foxes caused by, among others feeding with feedstuff of increased energy value.

Material and Methods
The investigations were performed at the "C" farm situated in the south eastern part of Poland. Stock of the basic pack comprised 500 females. The yearlings for pelting. Blood was collected by heart puncture from 60 minks "scan brown" fine brown variety. The 3 treetment groups were selected:K – kontrol, experimental D_1 and D_2 , the same number of males and females esch. Comparing the groups, each litter was divided randomly into three parts, regareling sex. It allowed to compare the genetic material among the groups. In blood serum of the animals examined activity of aspartic aminotransferase (AST), alanine selected for the experiment were meant aminotransferase (ALT), alkaline phosphatase (ALP), lactate dehydrogenase (LDH), and the level of urea, creatinine, glucose, bilirubin and uric acid.
The animals were fed properly balanced feedstuff. The feed composition was based on Cod, sprat x, poultry waste, horses stomach, meat - bone meal, animal fat, wheal meal – crude, bran, potatos boiled, water. It was also supplemented with pure fat animal. The doses were worked out with a computer program aid. The feedstuff energy value of 1 kg are enclosed at Table 1. The feed administred at all the groups contained vitamin-mineral premix GuyoFox at amount 0,10 kg/t feed (Garrido et al., 1996). The D_1 group was supplied with the antioxidant additive Rendox 0,02% of ready feed mass and preservative (sodium pyrosulphite) 0,28% ready feed mass.

Table 1. The quantitative composition of feed supplied to minks and energy value of 1 kg.

	01.08. - 15.09.			16.09. - 10.10.			11.10 - pelting		
	THE ENERGY VALUE (Kcal/kg)								
	K	**D_1***	**D_2**	**K**	**D_1***	**D_2**	**K**	**D_1***	**D_2**
	1700	1700	1700	1670	1670	1820	1690	1690	1900
	% EM								
PROTEIN	35.1	35.1	35.1	33.3	33.3	33.3	33.0	33.0	31.0
FAT	52.4	52.4	52.4	52.5	52.5	53.9	52.2	52.2	55.2
CARBO-HYDRATES (CH_2O)	12.5	12.5	12.5	14.2	14.2	12.8	14.8	14.8	14.8

** - This group was supplied with SODIUM PYROSULPHITE and RENDOX*

Both preparations were added to meat-fish materials kept in cold storage (Brandt, 1989, Kopczewski et al., 2000 and Slawoń, 1986) . Throughout the experimental period feed was provided ad libitum, with permanent water access.

About 2 weeks prior to the pelting, in D_2 group feed caloricity was reduced by 150 Kcal. owing to the animal anwillingness to have it.

In July and October, immediately after the feed was prepared 10 samples were taken at random from the mixer in order to perform the bacteriologic and microbiologic examinations. The laboratory examinations were performed according to the obligatory regulations PN-R-64791, PN-75/R-64787, PN-74A-74016 (Kopczewski et al., 2000; Kopczewski et al. 2001). The obtained results were analysed statistically computing arithmetic mean and standard deviation. Significance of differences between the means at 5% error risk of inference was verified with Student`s t-test (Microsoft Excel NT).

Results and Discussion

There have not been recorded any Salmonella rods in the examined feed, yet a greet contribution of poultry and poultry waste to animal feeding is hazardous for feed polution with various serotypes. Escherichia coli rods appeared to be isolate most frequently, its growth was obtined as mean numerous in 60% of the samples. There was also detected mean numerous growth of microbes Proteus g. in 80% of the studied samples and numerous growth of fungol colnies and moulds in all the samples.

The mean ALAT activity ranged from 120.28 to 224.80 U/l. This enzyme activity in serum increases not only at cell necrobiosis but at their damage as well (Garrido et al., 1996) . Alike, the AST activity exceeded the reference value oscillating from 95.66 to 133.49 U/l (Garrido et al., 1996; Heggset et al.,1999; Heggset 2000; Winnicka 1997) , whereas the ALP enzyme activity ranged from 60.20 U/l to 96.73 U/l. The results are presented in Table 2.

Elevated activity of this enzyme in serum may be connected with its discharge impairment through the bile ducts due to their occlusion, yet it is well known that an increase in ALP activity is more reliable for the evaluation of bone profile disturbances, vitamin D deficit and neoplastic processes (Winnicka 1997) . Liver is the main organ of the cholesterol and apolipoprotein biosynthesis. The cholesterol level - mean from three collections - in blood serum showed levels ranging from 5.82-6.21 nmol/l, yet contained within the limits (Garrido et al. 1996; Kopczewski et al. 2000; Sławoń 1986; Winnicka1997) .

Table. 2. The biochemical parameters in serum of mink (n=60).Means ($\bar{x}$) and Standard deviation (SD)

Collection		Bilirubin Mmol/l	Glucose mmol/l	Urea mmol/l	Uric acid mmol/l	Creatinine mmol/l	Cholesterol mmol/l	AP U/l	LDH U/l	AST U/l	ALT U/l
D_1	$\bar{x}$	3.67	6.39	5.95	0.21	69.46	5.82	74.39	1093.10	133.49	120.28
	SD	0.89	1.47	1.05	0.06	8.98	1.34	9.88	238.23	53.12	31.26
D_2	$\bar{x}$	2.05	7.71	6.10	0.21	56.03	6.21	96.73	883.27	132.3	224.80
	SD	0.29	1.95	2.61	0.03	3.93	1.98	17.77	425.53	28.30	80.30
K	$\bar{x}$	2.28	10.85	11.23	0.20	50.53	5.98	60.20	432.77	95.67	120.57
	SD	0.99	2.74	0.85	0.02	21.15	2.23	3.84	55.61	27.62	41.89

Alike, a mean bilirubin level in serum varied from 2.05-3.66 umol/l within the standard values and mean glucose level that reached 6,39-10,85 nmol/l at the standard up to 8,0 nmol/l (Winnicka1997).
Evaluating the renal profile, a urea level was detected, which is a final metabolite of protein metabolism in organism. Its concentration in blood serum depends on protein supply in a dietary unit, endogenic protein breakdown or excretory activity of kidneys. It is assumed that only at a glomerular filtration drop by over 50% there is recorded an increase of urea concentration in serum
(Winnicka1997) . A urea level was 5.94-11.23 nmol/l and was contained within the standards
(Sławoń 1986; Winnicka1997) . Creatinine present in serum is a metabolite of the skeletal muscles. It is discharged through the kidneys and useful for its excretory performance assessment
(Winnicka 1997) . The changes of a creatinine level are of a regular character with the lowest level recorded for 50.53 umol/l and the highest 69.46 umol/l not surpassing the reference values (Brandt, 1989; Winnicka 1997) . A uric acid level was very balanced and reached 0.203 mmol/l – 0.213 mmol/l being contained within the reference values (Brandt, 1989; Winnicka 1997) . Hypouricaemia may be conditioned by the enhanced excretion of uric acid through the kidneys, impaired reabsorption of this metabolite through the renal tubules as well as inborn deficiency of xanthine oxidase or an increase of its inhibitor level in organism (Brandt, 1989; Winnicka, 1997) . Summing up the results of the researches it may be stated that in the minks fed high energy feed there was detected the substantial growth of the AST and ALT activity. The rest parameters were contained within the values presented by the other authors (Brandt, 1989; Sławoń et al. 2000; Winnicka, 1997) .

References

Brandt A., 1989., (Ed.) Hematology and clinical chemistry of fur animals, 1st ed., 1989 Scientifur, pp 159.

Garrido G., Guzman M., Odriozola J. M.1996.: Effect of physical training on fatty acid metabolism in liver and skeletal muscle of rats fed four different highcarbohydrate diets. I. Nutr. Biochem., **7**, 348-355.

Heggset O., Skrede A.1999.: Nye Vitamin-Normer for Pelsdyrblad. Pelsdyoret Norsk Pelsdyrblad , **3**, 32-34.

Heggset O.,2000: Standardforlista 2000 Norsk Pelsdyrblad. Pelsdyoret Norsk Pelsdyrblad , **2**, 46-48.

Kopczewski A., Saba L., Bis-Wencel H., Sławoń J., Zoń A., Strzałkowski L., Zdunkiewicz T. 2000,: The results of microbiological and parasitological examinations of feed. Annls Univ.Mariae Curie-Sklodowska,sec.DD,55,315-31.

Kopczewski A.,Nozdryn-Płotnicki Z., Ondrašovič M., Bis-Wencel H., Saba L., Zoń A., Sławoń J., Strzałkowski L., Ulewicz W.2001.: The effect of feed with various hight-energy levels on anatomical and histopathological changes in arctic foxes. Folia Veterinaria , 45,3,134-138.

Sławoń J. 1986,: Nutrition of fox and mink.PWRiL, Warszawa, 27-30.

Sławoń J., Bis-Wencel H., Zoń A., Saba L., Kopczewski A., Wnuk W.,2000: Impact of high energy feedstuff on the growth of hair cover quality and anatomo-histopathological changes at polar fox.Zesz.Nauk.Przegl.Hod. 53,133-141.

Winnicka A.,1997,: The reference values of basic laboratory examinations in veterinary medicine. SGGW, Warszawa.

III – 17 RP

Correlation between liver fat and dry matter in mink (*Mustela vison*)

Clausen, T.N. & Sandbøl, P
Danish Fur Breeders Research Center, Herningvej 112 C, Tvis, DK-7500 Holstebro, Denmark;
tove.clausen@pfr.dk

Abstract

In our investigations on the protein requirement of mink in the growing period, we have often observed an increased fat content in the liver, when we reduced the protein / increased the fat content in the feed.

A chemical analysis of the fat content in the liver is relatively slow and expensive. We have used a semiquantitative test submerging liver samples into water and copper sulphate solutions with different specific gravities. On the basis of buoyancy in these liquids, liver samples were classified as containing > 34 % fat, 25 – 34 % fat, 13 – 25 % fat, or less than 13 % fat. The method is cheap but rather inaccurate. Furthermore the liquid cannot be used for more than a few liver samples before it has to bee replaced.

The dry matter content of fat is almost 100 percent, so we decided to determine a correlation between liver fat and liver dry matter. We took out liver samples from mink dying during October 2003. The livers were analysed for crude fat and dry matter content.

The results showed a very fine correlation between the dry matter and the fat content of the livers:

Liver fat, in percent = 1.15 * liver dry matter – 24.9 ($R^2 = 0.97$)

It is concluded that this method can bee used for a quick, cheap and acceptably precise evaluation of liver fat content.

Introduction

The liver function is highly influenced by the feed. Investigating the consequences of different feed compositions on growing mink kits, often makes it interesting to see if there are any fat infiltration in the livers. In our investigations on the protein requirement of mink in the growing period, we have often observed an increased fat content in the liver, when we reduced the protein / increased the fat content in the feed (Damgaard et al, 1994; Damgaard et al, 1998a; Damgaard et al, 1998b).

A chemical analysis of the fat content in the liver is slow and expensive. In order to screen the livers from many animals, we have searched for a fast and cheap method to determine liver fat content.

A semiquantitative test described by Herdt (1992), has been used for some years at the Research Center (Clausen, 1992; Damgaard et al, 1994; Damgaard et al, 1998a; Damgaard et al, 1998b). Liver samples were submerged into water and copper sulphate solutions with different specific gravities (1.000, 1.025 or 1.055). On the basis of buoyancy in these liquids, liver samples were classified as containing > 34 % fat, 25 – 34 % fat, 13 – 25 % fat, or less than 13 % fat. The method is cheap but rather inaccurate. Further the liquid cannot be used for more then a few liver samples before it has to bee replaced.

The dry matter content of fat is almost 100 percent. In this investigation we analysed dry matter and crude fat content of the livers and estimated the correlation between these two variables.

Material and methods

To the investigation we used liver samples from mink dying during October 2003. A total of 15 livers were chosen from their macroscopic appearance. 9 livers had a normal size and colour, 6 livers were very enlarged and yellow. From all livers we took out two equal samples from the same liver lobuli. One sample was analysed for crude fat (Stoldt fat, EU(98/64EØF)) and dry matter (104 ºC in 4 hours, EU(71/393/EØF)) content, at the Danish Fur Breeders Laboratory, and one sample was analysed for dry matter at the Research Center. At the Center we divided the liver sample into two parts each 2 - 4 grams and dried those at two different temperatures of respectively 80 ºC and 110 ºC. For practical reasons a drying time of 26 hours was chosen, as the samples were taken out in the morning and we then had the results the following day. The liver samples were mashed with a fork, and placed in small tin foil cubs before drying.

Results and discussion

Dry matter analyzed at the laboratory and at the Research Center was very equal. The best correlation was found between laboratory dry matter

and dry matter determined at the Research Center at 110 ºC for 26 hours (correlation coefficient 0.998, $p<0.0001$). Relationship between liver dry matter and crude fat content is shown in Figure 1.

Figure 1
Dry matter (percent) and the corresponding fat content (percent) of 15 mink livers. Regression line (y = 1,1523x - 24,903; R^2 = 0,973) with 95 percent confidence interval is shown.

The analyzed livers split up into two groups, one group with high dry matter above 58 percent and fat above 43 percent, and one group with dry matter below 43 percent and crude fat below 22 percent. The macroscopic appearance of the livers corresponded very well to the crude fat content; all the livers in the high fat group were big and yellow. A calculation based on an earlier investigation (Clausen, 1992) also showed a good relationship between liver fat and dry matter.

Conclusion

The results showed a very fine correlation between the dry matter and the crude fat content of the livers: Liver fat (percent) = 1.15 * liver dry matter (percent) – 24.9 (R^2 = 0.97)
It is concluded that this method can bee used for a quick, cheap and acceptably precise evaluation of liver fat content.

References

Clausen, T.N., (1992). Undersøgelse af fedtindholdet i leveren makroskopisk, kemisk, ved flydeprøve og sammenhæng til blodets ALAT indhold (Fat content of the liver evaluated macroscopically, chemically, by examination of the buoyancy in copper sulphate solutions and its connection to the content of ALAT in blood). Faglig Årsberetning 1991, Dansk Pelsdyravlerforening, 261-265.

Damgaard, B.M., Clausen, T.N. & P. Henriksen, (1994). Effect of protein and fat content in feed on plasma alanine aminotransferase and hepatic liver infiltration in mink. J. Vet. Med. A., 41, 620-629

Damgaard, B.M., Clausen, T.N. & H.H. Dietz, (1998a). Effect of dietary protein levels on growth performance, mortality rate and clinical blood parameters in mink (Mustela vison). Acta Agric. Scand. Sect. A., Anim. Sci., 48, 38-48.

Damgaard, B.M., Clausen, T.N. & C.F. Børsting, (1998b). Effect of dietary supplement of essential amino acids on mortality rate, liver traits and blood parameters in mink (Mustela vison) fed low protein diets. Acta Agric. Scand. Sect. A., Anim. Sci., 48, 175-183.

Herdt, T.H., Goeders, L, Liesman, J.S., Emery, R.S., 1983. Test for estimation of bovine hepatic lipid content. JAVMA, 182, 953 – 955.

III – 18 RP

Effects of feeding strategy on behaviour, physiological parameters and feed residues in mink females

Damgaard, B.M. & Hansen, S.W.
Danish Institute of Agricultural Sciences, Department of Animal Health and Welfare, Research Centre Foulum, P.O. Box 50, DK-8830 Tjele, Denmark. E-mail to corresponding author: BirtheM.Damgaard@agrsci.dk

Abstract
The effects of three feeding strategies were investigated in groups of 60 female mink from August to March. The three feeding strategies were: *ad libitum* feeding with a conventional diet from October to February, *ad libitum* feeding but with a substantial diet (high content of barley) from December to February, and restricted feeding with a conventional diet from October to February. The body weight was registered approximately every second week. Behavioural observations were made using focal sampling before and after feeding in December, January, February and March. Physiological parameters were measured in November, February and March. Feed residues were recorded individually on a two level scale (yes, no) each morning. The statistical methods included the proc mixed and proc genmod procedures in the SAS System. The results showed that restricted feeding increased stereotypic behaviour. It was demonstrated that it was possible to reduce the body weight of mink by feeding them a low energy feed without increasing the incidence of stereotypies. The feeding strategy had limited effects on physiological parameters. The interaction between feeding strategy, behaviour and occurrence of feed residues was demonstrated.

Introduction
In the wild and in production systems, mink increase their body weight from the summer period to the period of molting in October/November (Valtonen et al., 1995). The females that are to be used for breeding are selected in November among these often fairly fat females, and they are to be slimmed in order to best prepare them for flushing immediately before the mating season.
Restrictive feeding during the winter period results in an increase in the level of activity and, in particular, in the incidence of stereotypies (Bildsøe et al., 1991; Hansen et al., 2002; Houbak & Møller, 2000).
The purpose of this study was to examine the effects of traditional and alternative feeding strategies on the females’ weight and behaviour during wintertime on physiological parameters and on the probability of the occurrence of feed residues in the morning.

Materials and Methods
The study included 180 female mink (*Mustela vison*) of the colour-type ‘wildmink’ divided into three groups. The study was carried out from August to March. During the wintertime from December 22 to February 16 two groups (groups ADL and RE) were fed a conventional wet mink diet (dry matter (DM): 32.6%, metabolisable energy (ME): 5.16 MJ kg^{-1} wet diet (15.9 MJ kg^{-1} DM), distribution of ME with the following ratios of protein, fat, carbohydrate: 56% : 34% : 10%), and the third group (group SUB) was fed a substantial diet (DM: 35.2%, ME: 4.71 MJ kg^{-1} wet diet (13.4 MJ kg^{-1} DM), distribution of ME with the following ratios of protein, fat, carbohydrate: 49% : 25% : 26%). During the rest of the experimental period all three groups were fed conventional wet mink diet.
From October 17 to February 16 the three experimental groups were fed according to the following feeding strategies:

- Group ADL: ad libitum feeding – conventional wet mink diet
- Group SUB: ad libitum feeding – December 22 to February 16 substantial otherwise conventional wet mink diet
- Group RE: restrictive feeding – conventional wet mink diet

Each morning feed residues were recorded individually on a two level scale (yes, no). The females were weighed approximately every second week. Behavioural observations were made using focal sampling before and after feeding on December 14 and 28, January 24, February 14 and 28, March 7 and 28. The observer was placed 1 m

away from each cage section consisting of six cages. After 1 min waiting, the incidence and duration of certain behavioural patterns were recorded for 1 min. The first round of observations started 90 min before feeding and the second round started 15 min after feeding. On the basis of the behavioural observations from December 14 to February 14, the females were classified as stereotypic females (St-females) or as non-stereotypic females (Non-St-females).

Blood samples from 20 females per experimental group were collected in November, February, and March.

The packed cell volume (PCV) was determined in whole blood. The concentrations of urea, free fatty acids (FFA), and insulin were determined in plasma.

Statistical analyses were performed by means of the Statistical Analysis System (SAS) software (SAS Institute Inc., 1996). Effects and differences according to treatment, behaviour and time period were estimated using the MIXED and the GENMOD procedures in SAS.

Results and discussion

The planned approximated ad libitum feeding of the ADL and SUB groups was successful in that between 30% and 70% of the females left feed. As regards the RE group, the restrictive feeding strategy was successful and approximately 10 % of the females left feed. (Table 1)

The probability of the occurrence of feed residues was lower for the group fed restrictively (group RE) than for the two groups fed ad libitum (ADL and SUB groups) during the experimental period (Table 1, P=0.008). In the period from December 22 to February 16 the SUB group was fed a substantial diet and the ADL group a conventional diet but this difference in feed composition did not result in any differences in the probability of the occurrence of feed residues between the two groups (Table 1). Stereotypic females (St-females) had a lower probability of the occurrence of feed residues than non-stereotypic females (Non-St-females) in all experimental groups (Table 1, P=0.008). The probability of the occurrence of feed residues was lower in the period from October to December than in the period from December to February (Table 1, P<0.001).

On the basis of the behavioural classification 27% of the females in the groups fed ad libitum were classified as stereotypic females, while 53 % of the females fed restrictively were classified as stereotypic females.

In this experiment, the weight of stereotypic females was lower than the weight of non-stereotypic females. In the SUB group, the change from a conventional diet to a low energy diet resulted in a weight loss in all females independent of stereotypic classification.

Table 1.Probability of the occurrence of feed residues in stereotypic (St-females) and non-stereotypic (Non-St-females) females in the experimental groups ADL, SUB, and RE (*n*=60 per experimental group) in the periods from October 17 to December 22 and December 22 to February 16. Values are least square means and SE.

Experimental group	Period 1 October 17 – December 22		Period 2 December 22 - February 16		P-value Effects of:
	St-females	Non-St-females	St-females	Non-St-females	
ADL	35.1 % ± 12.2 % B	49.4 % ± 12.2 % A	42.3 % ± 13.1 % B	57.0 % ± 13.0 % A	Exp.group: <0.001 Stereotypy: <0.001 Exp.* Ste.: 0.008 Period: <0.001
SUB	35.5 % ± 11.1 % B	49.4 % ± 18.4 % A	42.7 % ± 10.5 % B	56.9 % ± 17.9 % A	
RE	4.3 % ± 18.4 % D	19.1 % ± 18.5 % C	5.8 % ± 19.7 % D	24.2 % ± 19.3 % C	

Mean values in the same period marked with different letters are significantly different (P<0.05).

Table 2.Packed cell volume (PCV) in blood and plasma concentrations of urea, free fatty acids (FFA) and insulin in stereotypic (St-females) and non-stereotypic (Non-St-females) females (*n*=60) in November, February and March. Values are least square means and SE.

Parameter/ Stereotypy	November 23-24	February 15-16	March 1-2	March 8-9	P-value Effect of:
PCV, %					Exp. group: 0.51
St-females	57.9 ± 0.99	55.5 ± 0.48	58.1 ± 0.53	55.2 ± 0.57	Stereotypy: 0.03
Non-St-females	58.3 ± 0.84	57.7 ± 0.42	59.0 ± 0.47	56.7 ± 0.49	Date: <0.001
Urea, mmol L^{-1}					Exp. group: 0.11
St-females	4.3 ± 0.24	6.3 ± 0.54	4.3 ± 0.28	6.4 ± 0.38	Stereotypy: 0.45
Non-St-females	4.2 ± 0.20	6.9 ± 0.45	4.5 ± 0.24	6.5 ± 0.33	Date: <0.001
FFA, mEqv L^{-1}					Exp. group: 0.54
St-females	0.48 ± 0.03	0.41 ± 0.03	0.51 ± 0.03	0.43 ± 0.03	Stereotypy: 0.32
Non-St-females	0.47 ± 0.03	0.46 ± 0.03	0.49 ± 0.02	0.51 ± 0.02	Date: 0.04
Insulin, mU L^{-1}					Exp. group: 0.18
St-females	14.3 ± 1.2	12.7 ± 1.2	8.7 ± 1.1	15.2 ± 1.1	Stereotypy: <0.001
Non-St-females	16.2 ± 1.0	17.3 ± 1.0	14.1 ± 1.0	20.3 ± 1.0	Date: <0.001

The PCV value in the blood and the plasma concentrations of urea, FFA and insulin were not affected by the feeding strategies (Table 2, P>0.05). Earlier investigations in mink females during the winter and reproduction periods have shown that mink females can maintain nutrient homeostasis within a wide variation in feed composition and feeding strategy (Børsting et al., 1998, Damgaard et al., 2003).
The blood PCV value was lower for St-females than for Non-St-females (Table 2, P=0.03), which may be correlated to the activity level and the fluid balance of the females. The PCV value was highest on March 1-2 at the end of the period with restrictive energy supply included in flushing (P<0.001). The activity level of the females did not affect the plasma concentrations of urea and FFA (Table 2, P>0.05). During the winter the plasma concentration of urea was lowest on March 1-2 which may be a result of a low energy supply and thereby a low protein metabolism in the beginning of March (Table 2, P<0.001). The plasma concentration of FFA was highest on March 1-2, which may be a result of the mobilisation of fat deposits at a time with low energy supply. The plasma concentration of insulin was lower for St-females than for Non-St-females (Table 2, P<0.001), and the concentration increased during the flushing period from February 15-16 to March 8-9 (Table 2, P<0.001). In earlier investigations in mink females, restrictive feeding followed by ad libitum feeding resulted in a significant increase in the concentration of insulin (Fink & Tauson, 1998; Tauson et al., 2000), which corresponds to the present results.

Conclusion

Restrictive feeding increased stereotypic behaviour. It was demonstrated that it was possible to reduce the body weight of mink females by feeding them a low energy diet without increasing the incidence of stereotypies. The probability of the occurrence of feed residues was affected by feeding strategy and stereotypic behaviour. The different feeding strategies had limited effects on metabolic and hormonal parameters. The metabolic parameters (FFA and urea) and the plasma concentration of insulin were influenced by the daily energy supply. The activity level of the females influenced the blood PCV value and the plasma concentration of insulin.

References

Bildsøe, M., Heller, K. E., Jeppesen, L. L., 1991. Effects of immobility stress and food restriction on stereotypies in low and high stereotyping female ranch mink. Behav. Proc. 25, 179-189.

Børsting, C. F., Damgaard, B. M., Fink, R., 1998. Effects of different energy supply prior to the breeding season on reproductive performance and metabolism in female mink. NJF-seminar no. 295, Bergen, Norway, 8 pp.

Damgaard, B.M., Børsting, C.F., Ingvartsen, K.L., Fink, R., 2003. Effects of carbohydrate-free diets on the performance of lactating mink (*Mustela vison*) and the growth performance of suckling kits. Acta Agric. Scand., Sect. A, Animal Sci. 53, 127-135.

Fink, R., Tauson, A.-H., 1998. Flushing of mink (*Mustela vison*): Effects on energy metabolism and some blood metabolites. Anim. Sci. 66, 277-284.

Hansen, S. W., Jensen, M. B., Pedersen, L. J., Munksgaard, L., Ladewig, J., Matthews, L., 2002. The type of operant response affects the slope of the demand curve for food in mink. Appl. Anim. Behav. Sci. 76, 327-338.

Houbak, B., Møller, S. H., 2000. Activity and stereotypic behaviour in mink dams fed *ad libitum* or restricted during the winter. VIIth Int. Sci. Congr. Fur Anim. Prod. Scientifur 24, No. 4, Vol. IV, pp. 146-150.

SAS Institute Inc. 1996. SAS/STAT*: Changes and Enhancements through Release 6.11. SAS Institute Inc., 1094 pp.

Tauson, A.-H., Fink, R., Forsberg, M., Lagerkvist, G., Wamberg, S., 2000. LH release in mink (Mustela vison). Pattern of the LH surge and effect of metabolic status. Reprod. Nutr. Dev. 40, 229-247.

Valtonen, M., Vakkuri, O., Blomstedt, L., 1995. Autumnal timing of photoperiodic manipulation critical via melatonin to winter pelage development in mink. Anim. Sci. 61, 589-596.

III- 19 RP

Regulation of lipid and glucose metabolism in the mink (*Mustela vison*) sequence analysis and development of molecular probes

Kathleen E. Glover and Kirsti Rouvinen-Watt
Canadian Centre for Fur Animal Research, Department of Plant and Animal Sciences, Nova Scotia Agricultural College, P.O. Box 550, Truro, Canada B2N 5E3

Abstract
Nursing sickness is an example of a metabolic disorder, which develops during the high energy demands of lactation and is characterized by a disruption in glucose homeostasis. We are examining the basic molecular mechanisms governing lipid and glucose metabolism in order to develop a better understanding of the regulation and relationship of body fat and blood sugar levels. Presently this research is focused specifically on the influences of obesity and dietary omega-3 fatty acid enrichment on glucose uptake by peripheral tissue and the deposition and mobilization of body fat in the mink. In the process of examining the molecular mechanisms governing lipid and glucose metabolism in the mink, we have developed laboratory procedures for the isolation and characterization of mink liver, skeletal muscle and adipose tissue mRNA. We will be evaluating changes in the gene expression of key enzymes and regulatory proteins of fat and glucose metabolism in response to dietary fatty acid composition and obesity. For the development of molecular probes for this work we have isolated and partially sequenced the mink acetyl-CoA carboxylase (ACC) using complementary DNA, which was prepared from reverse-transcribed mRNA. This sequence is highly conserved with respect to other mammalian species including human. Using similar methodology we have also partially sequenced the mink glucose transporter 4 (Glut4). In this paper we report these DNA sequences, which are the first for these enzymes in mink.

Introduction
Nursing sickness in the mink is a classic example of a metabolic disorder, which develops when the demands for lactation require extensive mobilization of body energy reserves. The animal enters a strongly negative energy balance and death usually follows soon after the first clinical signs (Clausen et al., 1992). The increasing age of the dam, followed by litter size and weight loss are major determinants for the development of nursing sickness. Although the ranch level epidemiology and the clinical pathology of nursing sickness are well documented, the etiology of this metabolic disorder remains unclear. The clinical symptoms of high blood glucose and insulin levels are commonly seen in the affected dams (Wamberg et al., 1992a), and it has been suggested that the development of the disorder is linked to disruption in glucose homeostasis (Børsting & Gade, 2000). Most recently it has been proposed (Rouvinen-Watt, 2003) that the underlying cause of mink nursing sickness may be acquired insulin resistance with obesity, omega-3 fatty acid deficiency or high protein oxidation rate as the key contributing factors.

Being an obligate carnivore, glucose homeostasis in the mink is directly dependent on protein and amino acid nutrition (Børsting & Gade, 2000). However, carbohydrate and protein metabolism are also linked to that of lipids (Frayn, 2001), which has received little consideration in the pathology of nursing sickness (Rouvinen-Watt, 2003). Long chain polyunsaturated fatty acids have been shown to regulate the expression of a variety of genes including major lipogenic enzymes (Clarke & Jump, 1994) such as fatty acid synthetase and acetyl-CoA carboxylase. In this context it is now well established that long chain polyunsaturated fatty acids can act as transcriptional activators of lipid metabolism (Tontonoz et al., 1995). More recently long chain omega-3 polyunsaturated fatty acids have been shown to attenuate the down-regulation of glucose transporter 4 mRNA which occurred when rats were fed high fat diets (Takahashi & Ide, 2000) indicating an involvement in glucose metabolism. Furthermore, these scientists were able to demonstrate in rats that dietary fish oil reduces blood glucose levels, improves glucose tolerance and increases insulin-stimulated glucose transport and metabolism in fat cells.

Very little is known about the molecular regulation of energy metabolism in carnivores. Using molecular

probes our objective is to examine the basic molecular mechanisms governing lipid and glucose metabolism in order to develop a better understanding of the regulation of blood sugar levels in the mink.

Material and Methods

RNA Isolation

Total RNA was extracted from mink (*Mustela vison)* adipose tissue using guanidinium isothiocyanate according to Chomczynski & Sacchi (1987) with the following modifications. A 1:1 mixture of water-saturated phenol and chloroform/isoamyl alcohol (24:1) was used for all phenol extractions. Shaking during the extractions was conducted for a minimum of 10 minutes and extractions were repeated until the interface was clear. The mink used in the study were of the standard black genotype.

Primer Selection

Forward and reverse primers for ACC were 5'- AGCACGCCAGGTTCTTATTG - 3' and 5' - GTGGTTGAGGTTGGAGGAGA - 3', respectively and were based on human ACC cDNA sequence. Forward and reverse primers for Glut4 were 5' - ATGTGTGGCTGTCGGATC - 3' and 5' - GAAGGTGAAGATGAAGAAG - 3', respectively and were based on canine cDNA sequence.

Reverse Transcription - Polymerase Chain Reaction

First strand cDNA was synthesized using 2 µg total RNA, oligo(dT) primers and MMLV reverse transcriptase following the manufacturer’s protocol (RETROscript, Ambion). Reverse transcription product (2-5 µl) was used as template for PCR amplification using Taq DNA polymerase, the primers described above and following the protocol provided with the RETROscript kit (Ambion). The ACC and Glut4 PCR products were gel purified, eluted and sequenced.

Results

Reverse transcription-polymerase chain reaction (RT-PCR) of RNA from mink adipose tissue using the ACC primers corresponding to nucleotides 3240-3259 and nucleotides 3605-3624 of human ACC1 mRNA (Mao, 2003) resulted in a cDNA fragment of approximately 385 base pairs. Sequence analysis was obtained for 308 base pairs (Figure 1) of the RT-PCR product, corresponding to nucleotides 3291-3598 of the human ACC1 cDNA. This sequence has been submitted to GenBank (Rouvinen-Watt & Glover, 2004a). The nucleotide sequence obtained shows greatest similarity to that of human (93%) followed closely by rat (92%), mouse (91%) and sheep (91%). The derived amino acid sequence of the mink ACC cDNA fragment (Rouvinen-Watt & Glover, 2004b) is given in Figure 2. All differences in nucleotide sequence among the five species compared were at positions of degeneracy in the genetic code and thus did not result in changes in amino acid sequence.

Reverse transcription-polymerase chain reaction (RT-PCR) of RNA from mink adipose tissue using the Glut4 primer pair corresponding to nucleotides 61-78 and nucleotides 447-465 of canine Glut4 mRNA (Christophe, 1999) resulted in a cDNA fragment of approximately 405 base pairs. Sequence analysis was obtained for 314 base pairs (Figure 3) of the RT-PCR product, corresponding to nucleotides 133-436 of the canine Glut4 cDNA. The nucleotide sequence obtained shows greatest similarity to that of horse (90%) followed closely by cow (89%), dog (88%) and human (88%). The derived amino acid sequence of the mink Glut4 cDNA fragment is given in Figure 4. Unlike the mink ACC cDNA fragment, nucleotide substitutions between the five species compared were not silent, resulting in differences between the amino acid sequences. There are seven amino acid differences between the mink and dog sequences but only three differences between mink and horse, cow or human. Two amino acid positions (15 and 28) contained phenylalanine, which appears unique to mink among these species comparisons. The Glut4 mink cDNA fragment will be sequenced again to confirm the nucleotide substitutions and derived amino acid sequence differences.

Discussion

Acetyl-CoA carboxylase catalyzes the carboxylation of acetyl-CoA to form malonyl-CoA, the donor of the two carbon units in the synthesis of long chain fatty acids. Activity of acetyl-CoA carboxylase is the rate- limiting step for *de novo* fatty acid synthesis in all organisms (Kim, 1997). Regulation of enzyme activity has been demonstrated at many levels including posttranslational short-term control via allosteric interactions with metabolites and reversible phosphorylation activated by hormones (Kim et al., 1989) and long-term control at the transcriptional level in response to various nutrients and hormones (Mao & Seyfert, 2002, Barber et al., 2001). It

is typically highly expressed in lipogenic tissues such as liver, adipose and lactating mammary gland and its regulation has been extensively studied in the rat, ovine, bovine and recently human tissue (Mao et al., 2003). In contrast to other species, *de novo* synthesis of fatty acids in mink mammary is thought to be minimal (Wamberg et al., 1992b). Tissue specific expression of ACC has been shown to be controlled at the transcriptional level by different promoters (Mao & Seyfert 2002). Very little is known about the structure and regulation of expression of the mink ACC gene in different tissues.

Insulin stimulates glucose uptake in fat and muscle by mobilizing a specific transporter known as glucose transporter 4 (Glut4), which enables glucose to be transported across the plasma membrane and into the cell. Glut4 is sequestered intracellularly in the absence of insulin, and is redistributed to the plasma membrane within minutes of insulin stimulation. This insulin sensitive, facilitated transport is critical for the control of blood glucose levels. The mechanisms that control Glut4 sequestration have not been fully discerned and are the subject of current research (Bogan et al., 2003; Lalioti et al., 2002). Although Glut4 is responsive to glucose, Glut4 gene expression also changes in response to dietary fat content and composition (Takahashi & Ide, 2000). As such Glut4 may represent a link between lipid and glucose metabolism. The relationship between dietary fat and glucose tolerance is being extensively investigated in the context of obesity and the development of diabetes in humans. Similar work in a carnivore such as the mink has not been conducted.

Both the ACC and Glut4 cDNA sequence data presented here represent the only such sequence available for the mink. The ACC and Glut4 RT-PCR products we have developed have been tested for their efficacy in ribonuclease protection assays and we will be using these products to study the transcriptional regulation of these enzymes. The goal of this work is to offer another dimension to on-going research concerning lipid and glucose metabolism in the mink and in particular the relationship of dietary fatty acid source to production related disorders, such as nursing sickness.

Acknowledgements

We thank Jennifer Fisher for her technical assistance and The Center for Applied Genomics, Hospital for Sick Children, Toronto, Ontario, for DNA sequencing. This work was supported by a Discovery Grant from NSERC to KRW and the Mink Farmer=s Research Foundation, Fur Commission USA.

References

Barber, M.C. 1994. *O. aries*. mRNA for acetyl-CoA carboxylase. GenBank direct submission. Accession X80045.

Barber, M.C., Pooley L. & Travers, M.T. 2001. Developmental regulation of alternatively spliced acetyl-CoA carboxylase-α mRNAs encoding isozymes with or without an eight amino acid domain upstream of the Ser-1200 phosphorylation motif in the mammary gland. J. Mol. Endocrinol. 27: 349:356.

Bogan, J.S., Hendon, N., McKee A.E., Tsao, T.S. & Lodish H.F. 2003. Functional cloning of TUG as a regulator of GLUT4 glucose transporter trafficking. Nature 425:727-733.

Børsting, C.F. & Gade, A. 2000. Glucose homeostasis in mink (*Mustela vison*). A review based on interspecies comparisons. Original Review. Scientifur 24:9-18.

Chomczynski, P. & Sacchi, N. 1987. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal. Biochem. 162:156-159.

Christophe, D. 1999. *Canis familiaris* mRNA for partial insulin-responsive glucose transporter, glut4 gene. GenBank direct submission. Accession AJ388533.

Clarke, S.D. & Jump, D.B. 1994. Dietary polyunsaturated fatty acid regulation of gene transcription. Annu. Rev. Nutr. 14:83-98.

Clausen, T.N., Olesen, C.R., Hansen, O. & Wamberg, S. 1992. Nursing sickness in lactating mink (*Mustela vison*). I. Epidemiological and pathological observations. Can. J. Vet. Res. 56:89-94.

Frayn, K.N. 2001. Adipose tissue and the insulin resistance syndrome. Proc. Nutr. Soc. 60:375-380.

Fukumoto, H., Kayano, T., Buse, J.B., Edwards, Y., Pilch, P.F., Bell, G.I. & Seino,S. 1989. *Homo sapiens* solute carrier family 2 (facilitated glucose transporter), member 4 (SLC2A4), mRNA. GenBank submission. Accession NM_001042.

Jose-Cunilleras, E., Hayes, K.A., Toribio, R.E., Mathes, L.E. & Hinchcliff, K.W. 2004. *Equis caballus* glucose transporter type 4 (GLUT4) mRNA, complete cds. GenBank direct submission. Accession AF531753.

Kang, M.-J., Jeong, Y.H., Lee, S.M., Park, H.Y., Yang, J.M., Mun S.C., Yoon, D.H. & Kim, D.M. 2003. *Bos taurus* GLUT4 mRNA, complete cds. GenBank direct submission. Accession AY458600.

Kim, K.H. 1997. Regulation of mammalian acetyl-coenzyme A carboxylase. Annu. Rev. Nutr. 17:77-99.

Kim, K.H., Lopez-Casillas, F., Bai D.H., Luo, X. & Pape, M.E. 1989. Role of reversible phosphorylation of acetyl-CoA carboxylase in long chain fatty acid synthesis. FASEB J. 3:2250-2256.

Lalioti, V.S., Vergarajauregui, S., Pulido, D. & Sandoval, I.V. 2002. The insulin-sensitive glucose transporter, GLUT4, interacts physically with Daxx. J. Biol. Chem. 277:19783-19791.

Lopez-Casillas, F. & Kim, K.H. 1988. *Rattus norvegicus* acetyl-coenzyme A carboxylase (Acac), mRNA. GenBank submission. Accession NM_022193.

Mao, J. 2003. *Homo sapiens* acetyl-CoA carboxylase 1 (ACC1) mRNA, complete cds; alternatively spliced. GenBank direct submission. Accession AY315627.

Mao, J., Chirala, S.S., & Wakil, S.J. 2003. Human acetyl-CoA carboxylase 1 gene: Presence of three promoters and heterogeneity at the 5'-untranslated mRNA region. Proc. Natl. Acad. Sci. 100:7515-7520.

Mao, J. & Seyfert, H-M. 2002. Promoter II of the bovine acetyl-coenzyme A carboxylase-α-encoding gene is widely expressed and strongly active in different cells. Biochim. Biophys. Acta 1576:324-329.

Rouvinen-Watt, K. 2003. Nursing sickness in the mink - a metabolic mystery or a familiar foe? Can. J. Vet. Res. 67:161-168.

Rouvinen-Watt, K., and K.E. Glover. 2004a. *Mustela vison* acetyl-CoA carboxylase mRNA, partial cds. Regulation of lipid metabolism in mink (*Mustela vison*). GenBank submission. Accession number AY494006.

Rouvinen-Watt, K., and K.E. Glover. 2004b. *Mustela vison* acetyl-CoA carboxylase mRNA, amino acid sequence. Regulation of lipid metabolism in mink (*Mustela vison*). GenBank submission. Accession number AAR86714.

Strausberg, R.L. 2003. *Mus musculus* acetyl-coenzyme A carboxylase, mRNA; partial cds. GenBank direct submission. Accession BC042725.

Takahashi Y., & Takashi, I. 2000. Dietary *n*-3 fatty acids affect mRNA level of brown adipose tissue uncoupling protein 1, and white adipose tissue leptin and glucose transporter 4 in the rat. Brit. J. Nutr. 84:175-184.

Tontonoz, P., Hu, E., Devine, J., Beale, E.G. & Spiegelman, B.M. 1995. PPARγ2 regulates adipose expression of the phosphoenolpyruvate carboxykinase gene. Mol. Cell. Biol. 15:351-357.

Wamberg, S. Clausen, T.N., Olesen, C.R. & Hansen, O. 1992a. Nursing sickness in lactating mink (*Mustela vison*). II. Pathophysiology and changes in body fluid composition. Can. J. Vet. Res. 56:95-101.

Wamberg, S., Olesen, C.R. & Hansen, H.O. 1992b. Influence of dietary sources of fat on lipid synthesis in mink (*Mustela vison*) mammary tissue. Comp Biochem. Physiol. 103:199-204.

Figure 1.Comparison of mink acetyl-CoA carboxylase (ACC) cDNA nucleotide sequence with sequence of human (Mao, 2003), mouse (Strausberg, 2003), rat (Lopez-Casillas & Kim, 1988) and sheep (Barber, 1994). Letters in bold denote differences between the species. Sequence alignment obtained from Blastn (NCBI).

1	taaccaagta gagtccatct tcctttcagc aattgacatg tacggacatc	mink
	taaccaagta gagtc**t**atct tcct**a**tcagc **t**attgacatg ta**t**ggacatc	human
	taaccaagta gagtc**t**atct tcct**a**tcagc **c**attgacatg ta**t**ggaca**c**c	mouse
	taaccaagta gagtccatct tct**ta**tcagc **c**at**c**gacatg ta**t**ggaca**c**c	rat
	caaccaagtc gagtc**t**atct tcct**g**tc**c**gc **c**attgacatg tacggacatc	sheep
51	agttttgcat tgagaactta cagaaactca tcttgtctga aacgtctatt	mink
	a**a**ttttgcat tgagaac**ctg** cagaaactca tc**c**t**a**tc**a**ga aac**a**tctatt	human
	agttttgcat tgagaac**ctg** cagaaactca tc**c**t**c**tc**g**ga aac**a**tctatt	mouse
	agttttgcat tgagaac**ctg** cagaaactca tc**c**t**a**tc**a**ga aac**a**tctatt	rat
	agtt**c**tgcat **c**gagaac**ctg** cagaaactca tcttgtc**c**ga aacgtc**g**att	sheep
101	tttgacgtcc taccaaactt cttctaccac agcaaccagg tagtgaggat	mink
	tttga**t**gtcc taccaaactt cttcta**t**cac agcaaccaag tagtgaggat	human
	tt**c**ga**t**gtcc t**c**ccaaactt **t**tt**t**taccac agcaaccagg t**g**gtgaggat	mouse
	tt**c**ga**t**gtcc t**c**ccaaactt **t**tt**t**taccac agcaaccagg t**g**gtgaggat	rat
	tttga**t**gtcc t**g**ccaaactt cttcta**t**cac agcaaccagg t**c**gtgaggat	sheep
151	ggcagctctg gaggtttatg ttcgaagggc ttatattgcc tatgaactta	mink
	ggcagctctg gaggt**g**tatg ttcgaagggc ttatattgcc tatgaactta	human
	ggcagctctg gaggt**g**tatg ttcgaagggc tta**c**attgcc tatgaact**c**a	mouse
	ggcggctctg gaggt**a**tatg ttcgaag**a**gc ttatat**c**gcc tatgagct**c**a	rat
	ggcagctctg gaggt**g**tatg ttcgaagggc ttatat**c**gcc tatgaactta	sheep
201	acagtgtaca gcatcgccag cttaaggaca acacctgtgt ggtggaattt	mink
	acag**c**gtaca **a**ca**c**cgccag cttaaggaca acacctgtgt ggtggaatt**c**	human
	acag**c**gtaca **a**ca**c**cgccag cttaaggaca acacctgtgt ggtggaattt	mouse
	acagtgtaca gcatcgccag cttaaggaca acacctgtgt ggt**a**gaattt	rat
	a**t**ag**c**gtaca **a**ca**c**cg**g**cag ct**g**aaggaca acacctg**c**gt ggtggaatt**c**	sheep
251	cagttcatgc tgcccacatc tcatccaaac agagggaaca tccccacgct	mink
	cagttcatgc tgcccacatc tcatccaaac agagggaaca tccctacgct	human
	cagttcatgc tgcccacatc **c**catccaaac agagggaaca tccccacgct	mouse
	cagttcatgc tgcccacatc tcatccaaac agagggaaca tccccacgct	rat
	cagttcatgc tgcccacatc **a**catccaaac agagggaaca tccccacgct	sheep
301	aaacagaa	mink
	aaacagaa	human
	aaacagaa	mouse
	aaacagaa	rat
	aaacagaa	sheep

Figure 2.Comparison of inferred protein amino acid sequence of mink acetyl-CoA carboxylase (ACC) with that of human, mouse, rat and sheep. Sequence references are given in Figure 1. Sequence alignment obtained from Blastp (NCBI).

```
 1   NQVESIFLSAIDMYGHQFCIENLQKLILSETSIFDVLPNFFYHSNQVVRMA         mink
     NQVESIFLSAIDMYGHQFCIENLQKLILSETSIFDVLPNFFYHSNQVVRMA         human
     NQVESIFLSAIDMYGHQFCIENLQKLILSETSIFDVLPNFFYHSNQVVRMA         mouse
     NQVESIFLSAIDMYGHQFCIENLQKLILSETSIFDVLPNFFYHSNQVVRMA         rat
     NQVESIFLSAIDMYGHQFCIENLQKLILSETSIFDVLPNFFYHSNQVVRMA         sheep

52   ALEVYVRRAYIAYELNSVQHRQLKDNTCVVEFQFMLPTSHPNRGNIPTLNR         mink
     ALEVYVRRAYIAYELNSVQHRQLKDNTCVVEFQFMLPTSHPNRGNIPTLNR         human
     ALEVYVRRAYIAYELNSVQHRQLKDNTCVVEFQFMLPTSHPNRGNIPTLNR         mouse
     ALEVYVRRAYIAYELNSVQHRQLKDNTCVVEFQFMLPTSHPNRGNIPTLNR         rat
     ALEVYVRRAYIAYELNSVQHRQLKDNTCVVEFQFMLPTSHPNRGNIPTLNR         sheep
```

Figure 3.Comparison of mink glucose transporter 4 (Glut4) cDNA nucleotide sequence with sequence of horse (Jose-Cunilleras et al., 2004), cow (Kang et al., 2003), dog (Christophe, 1999) and human (Fukumoto et al., 1989). Letters in bold denote differences between the species. Sequence alignment obtained from Blastn (NCBI).

1	ctgggcctgg	caggaatgtg	tggctgcgcc	atcctgatga	cttttgcgct	mink
	ctgggcctgg	c**g**ggaatgtg	tggctg**t**gcc	atc**t**tgatga	ct**g**t**g**gc**c**ct	horse
	ctgggcctgg	cagg**c**atgtg	tggctgcgcc	atc**t**tgatga	ct**g**t**g**gc**t**ct	cow
	ctgggcctgg	caggaatgtg	tggctg**t**gcc	atc**t**tgatga	cc**a**t**a**gc**c**ct	dog
	ctgggcctgg	c**g**gg**c**atgtg	tggctg**t**gcc	atcctgatga	ct**g**t**g**gc**t**ct	human
51	gcttctgctg	gagcgtgttc	ctgccatgag	cttcgtctcc	atcgtggcca	mink
	gcttctgctg	gagcg**a**gttc	c**a**gccatgag	ct**at**gtctcc	atcgtggcca	horse
	gcttctgctg	gagcg**g**gttc	c**a**gccatgag	ct**at**gtctcc	at**t**gtggcca	cow
	gcttctgctg	gagcg**gc**ttc	c**a**gccatgag	ct**a**cgtctcc	atcgtggcca	dog
	gct**c**ctgctg	gagcg**a**gttc	c**a**gccatgag	ct**a**cgtctcc	at**t**gtggcca	human
101	tctttggctt	tgtggcattc	tttgagatcg	gccccggccc	catcccctgg	mink
	tctttggctt	tgtggcattc	tttgagat**t**g	gccc**t**ggccc	catcccctgg	horse
	tctttggctt	**c**gtggc**c**ttc	tttgaaat**t**g	gccc**t**ggccc	catcccctgg	cow
	tctttggctt	tgtggc**c**ttc	tttgagat**t**g	gccc**a**ggccc	cat**t**ccctgg	dog
	tctttggctt	**c**gtggcattt	tttgagat**t**g	gccc**t**ggccc	cat**t**cc**t**tgg	human
151	ttcattgtgg	ctgaactgtt	cagccagggc	ccccggccag	cggccatggc	mink
	ttcat**c**gtgg	ctga**g**ct**c**tt	cagccaggg**a**	ccccg**c**ccg**g**	c**a**gccatggc	horse
	ttcat**c**gtgg	c**c**ga**g**ct**c**tt	cagccaggg**a**	ccccg**c**ccag	cggccatggc	cow
	ttcat**c**gtgg	c**c**ga**g**ctgtt	cagccagggc	ccccg**c**ccag	c**c**gccatggc	dog
	ttcat**c**gtgg	c**c**ga**g**ct**c**tt	cagccaggg**a**	ccccg**c**ccg**g**	c**a**gccatggc	human
201	tgtggccggc	ttctccaact	ggacgtgcaa	cttcatcatt	ggcatgggtt	mink
	tgtggc**t**ggc	ttctccaact	ggacgtgcaa	cttcatcatt	ggcatggg**c**t	horse
	agtggc**t**gg**g**	ttctccaact	ggacatgcaa	cttcatcatc	ggcatgggtt	cow
	cgtggc**t**ggc	ttct**g**caact	ggaca**a**gcaa	cttcatcatt	ggcatgggtt	dog
	tgtggc**t**gg**t**	ttctccaact	ggacg**a**gcaa	cttcatcatt	ggcatgggtt	human
251	tccagtatgt	ggcggaggct	atggggccct	acgtcttcct	tctcttcgcc	mink
	tccagtatgt	**c**gcgga**t**gct	atggg**t**ccct	acgtcttcct	tct**a**tt**t**gcg	horse
	tccagtatgt	ggcgga**t**gct	atggg**t**ccct	acgtctt**t**ct	tct**a**ttcgcg	cow
	tccagtat**a**t	**c**gcggangcc	atggggccc**t**	a**t**gtcttcct	tct**g**ttcgcg	dog
	tccagtatgt	**t**gcggaggct	atggggccct	acgtcttcct	tct**a**tt**t**gcg	human
301	gtcctcctgc	ttgg				mink
	gtcctcctgc	ttgg				horse
	gtcctcctgc	ttgg				cow
	gt**t**ctcctgc	t**c**g**c**				dog
	gtcctcctgc	t**g**gg				human

Figure 4.Comparison of inferred protein amino acid sequence of mink glucose transporter 4 (Glut4) with that of cow, horse, human and dog. Sequence references are given in Figure 3. Letters in bold denote differences between the species. Sequence alignment obtained from Blastp (NCBI).

1	LGLAGMCGCAILMTFALLLLERVPAMSFVSIVAIFGFVAFFEIGPGPIPWFIVAELFSQG	mink
	LGLAGMCGCAILMT**V**ALLLLERVPAMS**Y**VSIVAIFGFVAFFEIGPGPIPWFIVAELFSQG	cow
	LGLAGMCGCAILMT**V**ALLLLERVPAMS**Y**VSIVAIFGFVAFFEIGPGPIPWFIVAELFSQG	horse
	LGLAGMCGCAILMT**V**ALLLLERVPAMS**Y**VSIVAIFGFVAFFEIGPGPIPWFIVAELFSQG	human
	LGLAGMCGCAILMT**I**ALLLLER**L**PAMS**Y**VSIVAIFGFVAFFEIGPGPIPWFIVAELFSQG	dog
61	PRPAAMAVAGFSNWTCNFIIGMGFQYVAEAMGPYVFLLFAVLLL	mink
	PRPAAMAVAGFSNWTCNFIIGMGFQYVA**D**AMGPYVFLLFAVLLL	cow
	PRPAAMAVAGFSNWTCNFIIGMGFQYVA**D**AMGPYVFLLFAVLLL	horse
	PRPAAMAVAGFSNWT**S**NFIIGMGFQYVAEAMGPYVFLLFAVLLL	human
	PRPAAMAVAGF**C**NWT**S**NFIIGMGFQY**I**AXAMGPYVFLLFAVLLL	dog

III – 20 RP

The effect of protein level on N-balance in adult mink (*Mustela vison*)

Carsten Hejlesen, Danish Fur Breeders Research Center, Herningvej 112 C, 7500 Holstebro, Denmark. E-mail:ch.pfc@cfc.dk

Abstract

Diets containing 14.9, 19.0 and 26.7 % of metabolizable energy (ME) from protein and identical amino acid profile were fed ad lib. to adult male mink for 11 days. The average voluntary energy intake decreased (343, 306 and 261 kcal/day/animal, $p<0.0001$) as dietary energy content from protein increased. Daily energy requirement for maintaining constant weight was measured to 171 kcal ME/kg$^{0.75}$ at a temperature of 9.4 °C.

In the last 4 days, nitrogen (N) intake and N excretion (collection of urine and faeces) was measured and the N-balance calculated. N-balance was positive (0.08-0.24 g/day, NS) where as weight change was negative (÷1.6 - ÷8.3 g/day, NS). Regardless of dietary treatment the urinary N excretion declined linearly as digested N decreased. The conflicting positive N-balance and negative weight change was assumed reflecting an incomplete recovery of urinary nitrogen. If weight loss was regarded as either muscle or fat, the average urinary N-recovery was calculated to 86.3% and 92.9% respectively.

Oxidation of protein (OXP) per a calculated total heat production (HE) increased (11%, 16% and 19%, $p<0.05$) as dietary ME from protein increased.

Introduction

Basically the need for nutrients is a requirement for maintenance and a requirement dependant on a production (growth, foetus, milk, hair etc.). As nutrients, this is also the case for amino acids. The current dietary norm for 5 essential amino acids to mink in the growing-furring period were established in 1996 (Børsting & Clausen, 1996) and revised as well as including the 6th essential amino acid in 1998 (Børsting, 1998). In 2002 a minimum dietary content of all amino acids to mink in the winter- and reproduction periods were proposed (Hejlesen & Clausen, 2002). The results of these experiments cover periods which are quite different in regards to the production and are therefore a requirement for both maintenance and production. As the requirement for different kinds of production expectably is different, it would be opportune to know the requirement for maintenance. In the literature there have not been found any attempt to establish the dietary requirement of amino acids for maintenance to mink.

The requirement for amino acids for maintenance is a requirement for a continuous supply to the amino acid pool. Being a carnivore mink has a huge ability of gluconeogenesis, and therefore the importance of protein as energy source has been stressed (Chwalibog et al., 1998). On the other hand – the mink has a large glycolytic capacity (Fink, 2001).

The purpose of the experiment presented was to measure the N-balance in adult male mink fed low and decreasing levels of protein, with a constant level of fat and thereby a increasing level of carbohydrate. It was hypothesized that regardless of diet - a difference between nitrogen digested and nitrogen excreted in urine would reflect the accuracy in urinary nitrogen collection.

It was further-more the intention to evaluate 3 protein levels (with the same amino acid profile) in relation to the requirement of protein for maintenance by calculating the requirement of metabolizable energy for maintaining constant weight and by calculating N-balance. And finally to calculate oxidation of protein relative to total heat production.

Material and Method

The experiment was carried out with 5 adult male mink of the colour type Scanbrown, per diet. Three diets were composed to supply 14, 20 and 26% of metabolizable energy (ME) from protein and 55% from fat (table 1). The amino acid profile (Sandbøl et al. 2004) was the same in all 3 diets (amino acids relative to lysine (%): ala 107, arg 115, asp 133, cys 22, glu 241, gly 111, his 41, ile 74, leu 163, met 59, phe 85, pro 111, ser 89, thr 70, trp 22, tyr 67 and val 100). The animals were housed in metabolic cages designed for separate collection of faeces and urine (modified after Jørgensen, 1973). The temperature in the stable was 9.4 °C (8-11 °C) and relative

humidity 81% (65-83%). The experimental period was 11 days (16th to 27th of February 2004), with a 7 day preliminary period and a 4 day collection period (faeces and urine). The animals were fed 400 kcal per day (07[30]). Each day the feed consumption was measured (corrected for dry matter in left-overs), and in the collection period faeces was collected daily and stored at ÷18 °C until analysis (dry matter, crude ash, crude protein and crude fat). Urine was collected in bottles (500 ml) fitted under the funnels (collection period). The bottles were added 5% v/v H_2SO_4 (approx 60 ml) to keep pH low, and urine in the feeding tunnel was absorbed with N-free filtration paper and put in the storage bottle. Every day the collected urine (including drinking water wastage) was filled in bottles (2000 ml) which were stored at ÷18 °C until analysis (crude protein, pH). Animal weight change was recorded both in the preliminary and the collection periods.

Table 1. Composition of the diets used in the N-balance experiment with adult male mink.

Protein (% of ME)	**14.9%**	**19.0%**	**26.7%**
Industrial fish	2.8	3.8	4.8
Poultry offal	13.1	18.1	22.8
Slaughter offal	16.9	23.3	29.3
Barley/Wheat	3.8	5.3	6.6
Corn starch	16.7	10.7	5.2
Feather meal	0.8	1.2	1.5
Haemoglobin	0.2	0.3	0.4
Peas	2.9	4.0	5.0
Potato protein	1.5	2.0	2.5
Corn gluten	1.9	2.6	3.3
Protao	0.4	0.6	0.7
Soya bean oil	8.3	7.1	6.0
Lard	4.2	3.6	3.0
Methionine (DL)	0.3	0.4	0.5
Tryptophane	0.03	0.04	0.05
Lysine	0.02	0.03	0.03
Threonine	0.04	0.05	0.07
Vit. & Min.	0.3	0.3	0.3
Water	26.0	16.8	8.2
Analysis			
Dry matter, %	38.9	38.0	38.9
Kcal/100 g (wet)	201.6	193.9	188.0
ME from: Protein Fat Carbohydrate	 14.9 54.0 31.1	 19.0 53.8 27.2	 26.7 52.0 21.3

By measuring the apparent digestibility of crude protein, crude fat and crude carbohydrate the dietary content of ME was calculated per diet. Crude carbohydrate was calculated as dry matter ÷ content of crude ash, crude protein and crude fat.

When calculating the metabolic weight ($kg^{0.75}$), the absolute weight was calculated as average of the start and the final weight.

Quantitative oxidation of protein (OXP) was calculated by the formula

OXP, Kcal = (Urine N, g * 6.25 * 18.42)/4.1855, (Chwalibog 1992).

Results

For the entire experiment, the voluntary energy intake (ME) was significantly reduced as ME from protein increased resulting in a non-significant increase in weight loss (table 2).

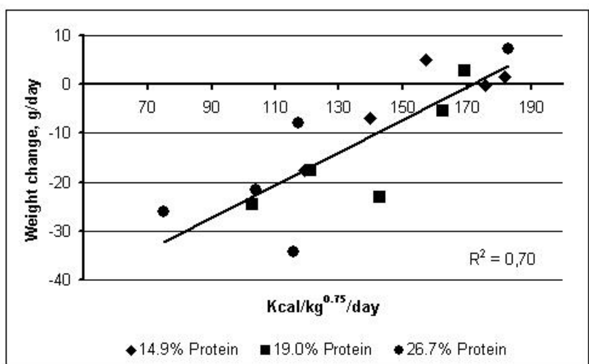
Table 2. Metabolic weight [1)] and daily energy ingested and weight change in the 11 day experimental period in the N-balance experiment with adult male mink.

Protein (% of ME)	**14.9**	**19.0**	**26.7**	**p<**
Metabolic weight, $kg^{0.75}$	2.22	2.19	2.19	NS
Kcal ingested	343^a	306^b	261^c	0.0001
Weight change, g	-3.6	-13.6	-16.5	NS

1) Metabolic weight calculated on the average of initial and final weight of the period.

The required metabolic energy for maintenance was 167, 177 and 169 kcal ME/$kg^{0.75}$ with a mean of all three groups of 171.4 kcal ME/$kg^{0.75}$ (R^2=0.703) (Figure 1).

Figure 1. Energy requirement (regardless of diet) for maintaining constant weight was 171 kcal ME/kg$^{0.75}$ per day (at 9.4 °C) (R^2=0.7) in the N-balance experiment with adult male mink.



In the collection period one animal was excluded from the analysis because it played with the drinking water nipple, which made analysis of N in urine impossible.

The pH in collected urine was 0.75 (± 0.14) which should prevent loss of volatile nitrogen.

The measured digestibility of crude protein (N) varied between 78.4 and 79.8 (NS), crude fat varied between 95.2 and 96.3 (NS) and crude carbohydrate increased from 74.5 to 84.9 with decreasing content of ME from crude protein. This was caused by the addition of the higher digestible starch when percentage of ME from crude protein was reduced.

Also in the collection period the voluntary energy intake (ME) decreased as the percentage of ME from protein increased, however the difference was not statistically significant (table 3). There was a slightly negative weight change in all three groups (NS). With the increasing ME from protein the digested N increased ($p<0.02$), but the degree of increment was reduced by the lower intake of feed. Urinary nitrogen increased ($p<0.005$) with increasing N content in the diets. The calculated nitrogen balance was positive with no difference (NS) between diets.

Discussion

Average daily voluntary intake of ME for the 11 day experimental period decreased as dietary protein content increased. This was also reported by Greaves & Scott (1960) for ad lib. fed adult cats on different dietary protein content. But it is in contrast to findings by Glem-Hansen & Chwalibog (1978). The reason to the reduced energy intake has not been identified, but it is unlikely to be an effect of palatability as the inclusion of the highly palatable ingredients (industrial fish, poultry offal and slaughter offal) increased with increasing dietary content of protein.

Table 3. Metabolic weight [1)] and daily weight change, energy intake and Nitrogen parameters in the 4 day collection period in the N-balance experiment with adult male mink.

Protein (% of ME)	**14.9**	**19.0**	**26.7**	**p<**
Metabolic weight, kg$^{0.75}$	2.21	2.17	2.14	NS
Weight Change, g	-4.4	-1.6	-8.3	NS
Kcal ingested	355	308	290	NS
Ingested N, g	2.4^{a}	2.6^{ab}	3.5^{b}	0.0230
Faecal N, g	0.5	0.6	0.7	NS
Digested N, g	1.88^{a}	2.07^{ab}	2.75^{b}	0.02
Urinary N, g	1.64^{a}	1.99^{ab}	2.60^{b}	0.01
N-Balance, g	0.24	0.08	0.15	NS
Recovery of urinary N, % (% of Digested N)	87.8	96.5	95.1	NS
Recovery of urinary N, % (Weight change=protein)	80.6	96.3	84.1	NS
OXP/HE[2)], %	11^{a}	16^{ab}	19^{b}	0.05

1) Metabolic weight calculated on the average of initial and final weight of the period.
2) Assuming weight change is fat.

Energy requirement for maintenance

By expressing weight change (g/day) as a function of digested energy per metabolic weight (kcal ME/kg$^{0.75}$) the energy requirement for maintenance was calculated to 171 kcal ME/kg$^{0.75}$ (R^2=0.703) (figure 1). This is above the 147.8 kcal ME/kg$^{0.734}$ found by Harper et al. (1978) however without reporting ambient temperature. Glem-Hansen & Chwalibog (1978) reported the requirement to 143 kcal ME/kg$^{0.75}$ at 20 °C, and a further requirement of 3.7 kcal ME/kg$^{0.75}$ per 1 °C decrease. Using this temperature dependant requirement results in a energy requirement for maintenance in this experiment corresponding to 132 kcal ME/kg$^{0.75}$ at 20 °C.

Even though there was only a minor discrepancy to the results by Glem-Hansen and Chwalibog (1978) they measured it as energy accretion per unit of energy digested. By expressing energy requirement as weight change per unit of energy digested it is assumed that the relative body composition (protein, fat and glycogen) remains constant. Further more there is a potential risk of a bias when weighing the animals. As the animals have unrestricted access to feed and drinking water it is unknown whether the animals just ate, drank or defecated at the time of weighing.

Recovery of urine N

The N-balance (digested N ÷ urinary N) was positive for all three groups (0.08 – 0.24 g/day) (table 3). Calculating with 25% protein in “muscle” gain it corresponds to a “muscle” gain of 2.0 – 6.1 g per day.

Even though mink has the ability to reduce oxidation of protein when protein supply is reduced (Chwalibog et al., 1998; Fink, 2001 & 2003; Tauson, 2000) protein accretion is unlikely when weight change is negative.

So, even with the above mentioned reservation to weight change, a muscle gain is in conflict to the measured negative weight change.

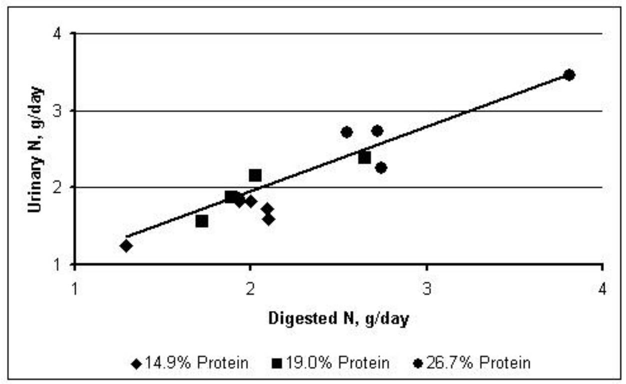
The reason to the discrepancy is presumably a recovery rate of urinary nitrogen below 100%. Using precollected urine (not stabilized with acid) Elnif (1992) simulated urination and measured recovery of nitrogen of 62% to 71%. A incomplete recovery (72%) in urine was also demonstrated by Wamberg et al. (1996) with female mink. Probably because of the female anatomy and behaviour when excreting they too found 6% of the urinary excretion in the collected faeces.

Assuming the weight change was fat, the recovery of urinary nitrogen (100 * Urinary N/Digested N) in this experiment was 88% to 97% whereas it was 81% to 96% if the weight change was regarded as protein (table 3). These recovery rates are higher than reported by Elnif (1992) and Wamberg et al. (1996). This could be an effect of collecting wastage water and urine in the same bottle. Wastage water might function as a continuously partly washing of the collection funnels. The possible negative effect is however that the dilution of the urine can have a magnitude which jeopardizes the determination of urinary nitrogen.

N-balance

For animals at maintenance the urinary N excretion decrease linearly when digested N decreases as long as the digested quantity of N meets the requirement of the animal. If it doesn’t, N-balance is negative i.e. urinary excretion exceed digested quantity. The mean N-balance for the animals fed 14.9 percent of ME from protein was not negative or lower than for the other two groups. And when urinary N excretion is plottet against digested N (figure 2) individual data for animals fed 14.9% of ME from protein fits well with the regression line for animals fed 19.0 and 26.7% of ME from protein.

Figure 2. Urinary N excretion declines as digested N decreases. Individual results for 14.9 % of ME from protein fits well (and are not above) the regression line for 19.0 and 26.7 % of ME from protein in the N-balance experiment with adult male mink.



This indicates that 14.9 % of ME from protein and the used amino acid profile did fulfil the animals requirement for maintenance.

OXP, % of HE

Assuming the weight loos is fat (contributing 9.5 kcal heat energy/g) and weight gain is fat (contributing 2.4 kcal heat/g (20% heat production in fat synthesis from fat and carbohydrate) and assuming total heat production is equal to digested energy corrected for contribution from oxidation or synthesis of fat the heat production from protein oxidation would account for 11.3%, 16.2% and 19.3 % of the total heat production in the 3 different diets (table 3).

It has been shown, that mink has the ability to reduce oxidation of protein if protein supply is reduced either by reducing the dietary protein content (Fink, 2001 & 2003 and Tauson, 2000) or by reducing the dietary quantity (Chwalibog et al., 1998).

Even though the heat production is calculated, the data presented here strongly suggest, that the oxidation of protein can be reduced to less than found by Chwalibog (1998) for non productive females, Tauson (2000) and Fink (2001 & 2003) for lactation females. Their higher measured oxidation of protein (% of HE) is most likely a result of diets containing more of the ME as protein. A low supply of protein does however require a sufficient supply of carbohydrates to maintain glucose homeostasis (Fink, 2001).

Conclusion

The recovery of urinary nitrogen was calculated to a mean of 92.9% if weight change was regarded as fat, and 86.3 % if weight change was regarded as protein.

Heat energy from oxidation of protein in % of total heat production was reduced when the protein content of the diet was lowered from 26.7% to 19.0% and 14.9 % of ME.

The data indicated that 14.9 % of ME from protein (and the used amino acid profile) did fulfil the animals protein requirement for maintenance, corresponding to 0.85 g digestible N/kg$^{0.75}$ per day.

References

Børsting, C.F. & T.N. Clausen. 1996. Requirement of essential amino acids for mink in the growing-furring period. Proc. VI Int. Sci. Cong. Fur Anim. Prod. (Eds. A. Frindt and M. Brzozowski), Warsaw, Poland. Anim. Prod Rew., Appl. Sci. Rep., 28, 15-24

Børsting C.F. 1998. Ernæringens indflydelse på skindkvaliteten. Intern Rapport Nr 111, Danmarks JordbrugsForskning. 55-62.

Chwalibog A., K. Jakobsen, S. Henckel & G. Thorbek. 1992. Estimation of quantitative oxidation and fat retention from carbohydrate, protein and fat in growing pigs. Journal of Animal Physiology, A Animal Nutrition. 68, 123-135.

Chwalibog A., A-H. Tauson, R. Fink & G. Thorbek. 1998. Oxidation of substrates and lipogenesis in pigs (*Sus scrofa*) mink (*Mustela vison*) and rats (*Ratus norvegicus*). Thermochimica Acta, 309, 49-56.

Elnif J. 1992. Accuracy og nitrogen balance measurements in adult mink. Norwegian Journal of Agricultural Sciences. Supp. 9, 254-260

Fink R. 2001. Nutrient and energy metabolism in the lactation mink (Mustela Vison). PhD Thesis. Department of Animal Science and Animal Health, The Royal Veterinary and Agricultural University, Copenhagen. pp. 130

Fink R., A-H Tauson, A. Chwalibog, N.B. Kristensen & S. Wamber. 2003. Effects of low protein supply on energy metabolism and milk production of the mink (*Mustela vison*),. Progress in research on energy and protein metabolism. EAAP publication No. 109, Rostock-Warnemünde, Germany, 13-18 September 2003, 163-166.

Glem-Hansen N. & A. Chwalibog. 1978. Minkens energibehov til vedligehold. NJF meeting 1978, 9.-11 . October, Helsingør, pp 10.

Greaves J.P. & P.P. Scott. 1960. Nutrition of the cat. 3. Protein requirement for nitrogen equilibrium in the adult cats maintained on a mixed diet. British Journal of Nutrition, 14, 361-369

Hejlesen, C. & T.N. Clausen. 2002. Energy Distribution in Mink Feed in the Winter and Reproduction Periods. Annual Report, 2001, 69-75. Danish Fur Breeders Research Center. Holstebro Denmark.

Jørgensen G. & Glem-Hansen N., 1973. A Cage designed for Metabolism- and Nitrogen Balance Trials with Mink. Acta Agriculturæ Scandinavica, 23, 3-4

Sandbøl P, T.N. Clausen & C. Hejlesen. 2004. Ideal Protein for Mink (*Mustela vison*) in the

Growing and Furring Periods. VIII International Scientific Congress in Fur Animal Production. 15-18 September 2004. The Netherlands. This publication.

Tauson A-H., R. Fink & A. Chwalibog. 2000. Protein as an energy source for lactating mink (*Mustela* vison) estimated by breath test measurements of decarboxylation of 1-^{13}C-labelled leucine. In Energy metabolism in animals. Proceedings of the 15. Symposium on energy metabolism in animals. Snekkersten 2000. 261-264

Wamberg S., A-H Tauson & J. Elnif, 1996. Effects of feeding and short-term fasting on water and electrolyte turnover in female mink (*Mustela vison*). British Journal of Nutrition. 76, 711-725.

III – 21 RP

Bacterial protein produced on natural gas as a protein source in dry diets for the growing-furring blue fox

Anders Skrede and Øystein Ahlstrøm
Department of Animal and Aquacultural Sciences, Agricultural University of Norway, P.O. Box 5003, N-1432 Ås, Norway
Corresponding author: Anders Skrede (anders.skrede @iha.nlh.no)

Abstract
Bacterial protein meal (BPM) grown on natural gas as the carbon and energy source, and ammonia as the nitrogen source, was evaluated with respect to digestibility, feed intake, growth and fur characteristics in the growing-furring blue fox (*Alopex lagopus*). The BPM was produced by continuous aerobe bacterial fermentation, using methanotrophic bacteria (*Methylococcus capsulatus* Bath, *Alcaligenes acidovorans*, *Bacillus brevis* and *Bacillus firmus*), and contained approximately 70% crude protein and 10% lipids. Four extruded dry diets containing 0, 4, 8 and 12% BPM, replacing fishmeal, soybean meal and meat meal in a 3:1:1 ratio on a crude protein basis, were fed to groups of 20 weaned blue fox cubs (10 males and 10 females) from August 8 to December 5. The highest level of BPM corresponded to 30% of total dietary crude protein. Digestibility studies showed no significant effect of increasing levels of BPM on digestibility of crude protein, fat or carbohydrate. The growth experiment was carried out without health problems and there was no mortality. Body weights and weight gain were not significantly different among the diets, but there was a tendency (P<0.10) towards increased body weight gain with increasing level of BPM. Feed conversion appeared to be slighly improved with increasing dietary inclusion of BPM. Skin size and fur characteristics were not significantly affected by dietary treatment. It is concluded that bacterial protein meal produced from natural gas-utilising bacteria seems to be a suitable alternative protein source for growing-furring blue foxes.

Introduction
The idea of using microorganisms as sources of protein in animal nutrition is not a new one. Single cell protein sources (bacteria, yeast, algae or fungi) have high protein content and the potential yield per unit area may be very high. Thus the rapid growth rate and high protein content are well known advantages of bacteria for protein production (Roth, 1980; Stringer, 1982).

Recently, a commercial scale production plant with an annual capacity of 10 000 metric tons of bacterial protein meal, has been built at Tjeldbergodden, Norway. The production of bacterial protein meal (BPM) is founded on continuous aerobe fermentation using natural gas as the carbon and energy source, and ammonia as the nitrogen source for protein synthesis. Briefly, the BPM consists of the killed and spray-dried biomass of four different naturally occurring bacteria, *Methylococcus capsulatus* (Bath), *Alcaligenes acidovorans*, *Bacillus brevis*, and *Bacillus firmus* (Skrede et al., 1998). The BPM is a reddish/brownish meal containing approximately 96% dry matter, 70% crude protein and 10% fat (Skrede et al., 1998). The average apparent amino acid digestibility of BPM is about 80% in several animal species, including mink, Atlantic salmon, pigs and young chicks, typically with high digestibilities of lysine and arginine, and rather low digestibility of cysteine (Skrede et al., 1998). Recent studies have shown that BPM is suitable as a major protein source in diets for weanling and slaughter pigs, broiler chicken and Atlantic salmon (Øverland et al., 2001; Skrede et al., 2003; Berge et al., 2004; Storebakken et al., 2004).

The potential of BPM as an ingredient of diets for foxes has not been extensively investigated. In the present study, experimentally produced BPM gradually replaced fishmeal, soybean meal and meat meal in dry diets for blue foxes during the growing-furring period. The aims were to study if this replacement influenced nutrient digestibility, feed intake, growth, survival, or fur characteristics.

Material and methods
The study was carried out at the Department of Animal and Aquacultural Sciences, The Agricultural University of Norway, Ås, Norway.

Four extruded dry diets, containing 0% BPM (BPM0) as a control diet, 4% BPM (BPM4), 8%

BPM (BPM8) or 12% BPM (BPM12), were used. The diets were formulated to contain equal levels of crude protein, fat and carbohydrate, and to cover requirements for methionine + cysteine and other essential amino acids for foxes. The highest level of BPM corresponded to 30% of total dietary crude protein. Increasing levels of BPM was balanced by a reduction in fishmeal, meat meal, and soybean meal (SBM) protein, approximately at a 3:1:1 ratio. The chemical composition and amino acid profile of the protein sources are given in Table 1.The BPM (“BioProtein”) was supplied by Norferm AS, Stavanger, Norway. Other dietary ingredients were obtained from commercial suppliers.

Table 1. Proximate composition (g kg^{-1}) and amino acid composition (g/16g N) of the protein sources (BPM= bacterial protein meal; SBM= soybean meal).

	BPM	Fish-meal[a]	SBM[b]	Meat meal[c]
Dry matter	958	927	911	952
Crude protein (Nx6.25)	697	717	484	604
Crude fat	102	96	15	94
Ash	80	117	67	198
Amino acids				
Cysteine[1]	0.61	0.97	1.51	0.50
Methionine	2.91	2.96	1.26	1.99
Aspartic acid	8.88	9.56	11.47	7.69
Threonine	4.65	4.25	3.92	3.62
Serine	3.69	4.30	5.82	3.85
Glutamic acid	10.57	12.91	20.16	13.53
Proline	4.18	4.10	5.75	7.59
Glycine	5.16	5.81	4.65	11.87
Alanine	7.15	6.06	4.83	8.55
Valine	6.01	5.29	5.51	4.88
Isoleucine	4.69	4.77	5.18	3.73
Leucine	7.72	7.86	8.24	6.83
Tyrosine	3.71	3.25	4.09	2.99
Phenylalanine	4.28	4.13	5.36	3.93
Histidine	2.33	2.56	2.92	2.51
Lysine	5.93	7.99	5.74	6.59
Arginine	6.31	6.07	7.45	6.73
Tryptophan	2.08	0.93	1.13	*1.10

[1]Cysteine and cystine. Table value.*
[a]Norseamink, Norsildmel, Bergen, Norway.
[b]Denofa AS, Fredrikstad, Norway.
[c]Norsk Protein, Hamar, Norway

Table 2. Ingredient composition (g kg^{-1}) and chemical content of extruded blue fox diets containing from 0 – 12 % of bacterial protein meal (BPM).

Diet	BPM0	BPM4	BPM8	BPM12
Bacterial protein meal	0	40	80	120
Fish meal[a]	155	135	115	95
Soybean meal[b]	114	104	94	84
Meat meal[c]	104	94	84	74
Carbohydrate mix[d]	436	436	436	436
Beet pulp	40	40	40	40
Soybean oil	148	148	148	148
Vit./min. mix[e]	3	3	3	3
Calculated content (g kg^{1})				
Dry matter	920	921	922	923
Crude protein	270	272	274	276
Crude fat	191	190	190	189
Ash	56	54	53	51
Total carbohydrate	403	405	405	407
Analysed content (g kg^{-1})				
Dry matter	939	933	917	933
Crude protein (Nx6.25)	274	270	275	278
Crude fat	191	203	185	203
Ash	68	65	62	65
Total carbohydrate	406	395	395	387

[a]Norseamink, Norsildmel, Bergen, Norway.
[b]Denofa AS, Fredrikstad, Norway
[c]Norsk Protein, Hamar, Norway. [d]FK-Carbo, Felleskjøpet Øst Vest, Oslo, Norway. [e] Norsk Mineralnæring, Hensmoen, 3516 Hønefoss, Norway, containing per kg: Fe 2000 mg, Cu 125 mg, Mn 750 mg, Zn 1000 mg, vit. A 200 000 IE, vit. D_3 20 000 IE, vit. E 50 000 mg, vit. B_1 15 000 mg, vit. B_2 3000 mg, vit. B_6 3000 mg, vit. B_{12} 20 mg, Calcium-d-pantothenate 3 000 mg, niacin 5000 mg, folic acid 300 mg, and biotin 30 mg.

The diets were produced at Centre for Feed Technology, Ås, Norway. The ingredient composition and contents of the experimental diets are shown in Table 2. Each batch was mixed in a Dinnisen twin shaft high-speed mixer, conditioned in a Milltenz singler shaft pre-conditioner (501S, Millband Technology LTD, Auckland, New Zealand), and extruded at defined conditions using a twin-screw Bühler extruder (EX-50/134 L 90 kW, Uzwil, Switzerland). The diets were dried to approximately 93% dry matter in a Milltenz counterflow dryer (VC010 Gas, Millband

Technology Ltd, Auckland, New Zealand before soybean oil was added using a Dinnisen vacuum coater (Oscar Menger, Sevenum, Holland). Prior to bagging the diets were cooled in a Münch counter flow cooler (Münch-Edelstahl GMBH, Hilden, Germany).

A digestibility experiment was carried out from October 27th to November 10th, using three male blue foxes per dietary treatment. The animals were kept at approximately normal hours of day length and a temperature of about 16 °C. The individual cages were provided with devices for controlled feeding and separate collection of feces and urine. The experimental period consisted of a 3-day preliminary period and a 4-day fecal collection period. Individual samples of feces were bulked, homogenized and freeze dried pending analysis.

In the main experiment, each diet was given to 20 weaned blue fox cubs, 10 males and 10 females, with an initial body weight of approximately 2.8 kg. The animals were allocated to four groups according to body weight and genotype. The groups were randomly assigned to treatment. The experiment started August 8th, when the animals were approximately two months old, and terminated December 5th. The animals were kept under conventional farm conditions, using two animals of the same sex in each cage. The dry diets were added water in proportion 1/3 feed to 2/3 water. The wet mix was allowed to swell to a dough consistency for 14-15 h before feeding on boards once a day. Ad libitum feeding was adopted. The animals were given free access to water through a semi-automatic watering system.

The animals were weighed individually at the start of the experiment, and at 4-week intervals thereafter. Feed consumption was recorded on a group basis as feed offered minus feed rejected. Evaluation of fur characteristics was carried out on dried and undressed skins by experienced graders.

Analyses of dry matter, crude protein (Kjeldahl-N x 6.25), crude fat (HCl/ethyl ether extract) and ash, were carried out by the Laboratory of Analytical Chemistry, Agricultural University of Norway, using the methods of AOAC (1980). Crude carbohydrate was determined using difference calculation. Apparent values of total tract digestibility were determined as the average of three individual determinations.

Statistical analysis was carried out using the GLM procedure of SAS (1990). The level of significance was set at $P<0.05$.

Results and discussion

Digestibility experiment

All diets were well eaten by the animals and there were minor differences in fecal consistency. There were no significant differences between diets in digestibility of crude protein, fat or carbohydrate (Table 3). Linear regression analysis showed no significant effect of increasing levels of BPM on nutrient digestibility.

Table 3. Average digestibility of main nutrients (%) in experiment with diets containing from 0 to 12 % of bacterial protein meal (BPM) for blue foxes. Standard deviations in parentheses.

Diet	BPM0	BPM4	BPM8	BPM12
Crude protein	94.5 (1.5)	92.4 (1.5)	92.8 (0.7)	93.0 (1.6)
Crude fat	96.4 (0.5)	96.2 (0.5)	95.9 (0.6)	96.7 (0.5)
Crude carbohydrate	68.6 (5.1)	62.9 (2.2)	64.9 (4.7)	69.3 (3.6)

No significant differences were found.

Production experiment

All animals accepted the diets well throughout the entire experimental period. Increasing dietary levels of BPM appeared to have no effect on palatability. No signs of any health problems were seen during the experiment and there was no mortality.

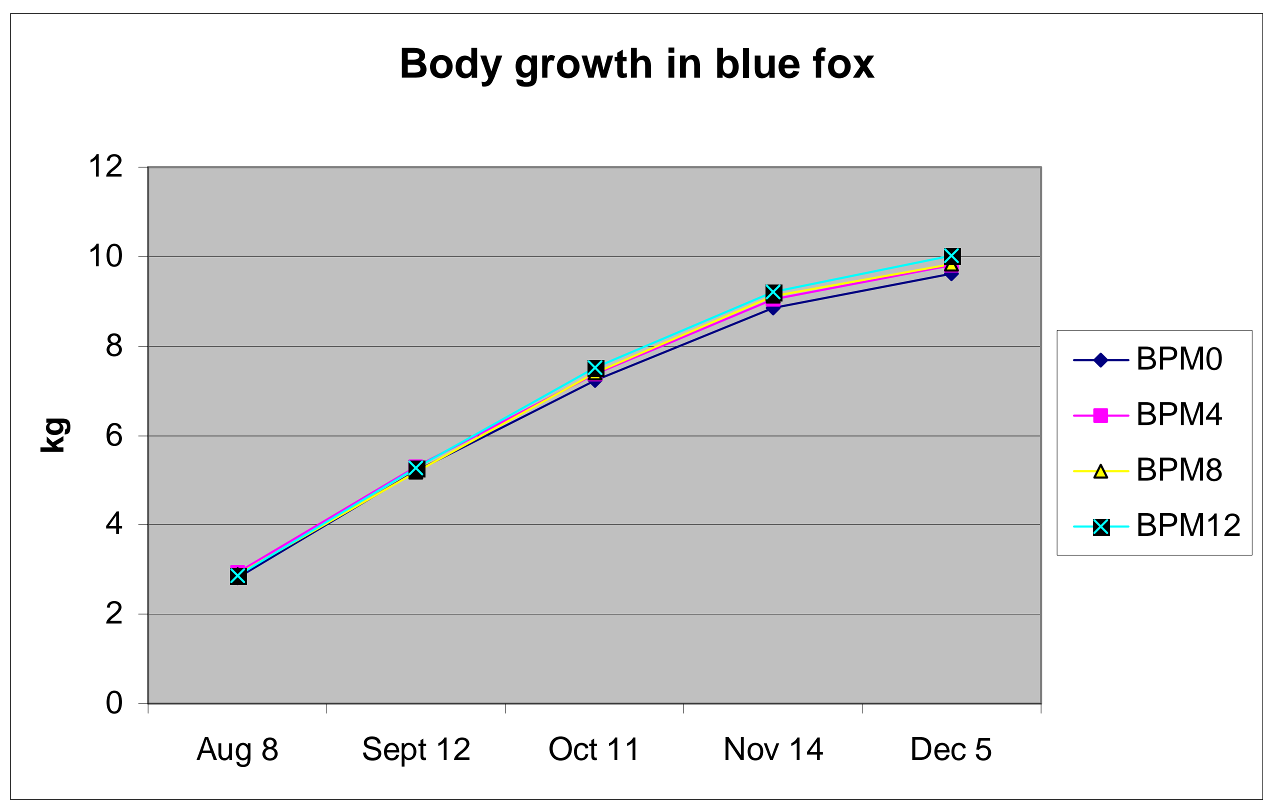
Data on feed consumption are shown in Table 4, and growth data are presented in Table 4 and Figure 1. The animals revealed generally satisfactory weight gain, considering the genetic potential of the blue fox strain used in the present experiment. There was a tendency ($P>0.05$) towards enhanced body gain with increasing levels of BPM. This slight increase in growth was achieved without increase in feed consumption, indicating an improvement of feed conversion due to the feeding of BPM (Table 4). Similar results have been obtained in studies with broiler chicken (Skrede et al. 2003). A possible mechanism affecting feed conversion may be related to the amino acid profile of BPM, especially the high level of tryptohan (Table 1). Tryptophan is a precursor of the neurotransmitter serotonin, which indirectly may influence energy requirement by modulating animal behavior as shown in studies with silver foxes (Rouvinen et al., 1999).

Table 4. Overall body weight gain, feed consumption, and feed conversion from August 8th to December 5th in blue foxes fed diets containg from 0 to 12 % of bacterial protein meal (BPM). Standard deviation in parentheses.

Diet	BPM0	BPM4	BPM8	BPM12
Overall gain (kg)	6.79 (1.18)	6.87 (1.36)	6.95 (1.03)	7.16 (1.31)
Average dry feed consumption per animal (g/day)	295.8	294.6	295.2	293.8
Feed conversion rate (kg feed/kg body gain)	5.18	5.10	5.05	4.88

No significant differences in body weight gain were found.

Figure 1. Body growth in blue foxes fed diets containg from 0 to 12 % of bacterial protein meal (BPM).



Skin size, measured by length and weight, and the evaluation of fur quality characteristics showed no significant differences between treatment groups (Table 5). Thus, fur quality was not influenced by dietary inclusion of BPM. However, this should be confirmed in future experiments with a larger group size than used in the present study.

It was concluded from the present study that bacterial protein meal produced from natural gas-utilising bacteria seems to be a suitable alternative protein source for the growing-furring blue fox.

Acknowledgement

The studies were supported by grant # 143196/140 "Protein produced from natural gas - a new feed resource for fish and domestic animals" from the Research Council of Norway, and by a grant from Norferm AS, Stavanger, Norway.

Table 5. Average skin length and weight, and fur characteristics in blue foxes fed diets containg from 0 to 12 % of bacterial protein meal (BPM). The figures represent 50% males and 50 % females. Standard deviation in parentheses

Diet	BPM0	BPM4	BPM8	BPM12
Skin length (cm)	109.0 (6.1)	109.0 (4.4)	109.8 (3.8)	109.2 (5.7)
Skin weight (g)	636.1 (80.6)	624.5 (75.5)	674.2 (65.5)	664.1 (96.4)
Colour[1]	5.5 (1.2)	5.7 (1.5)	5.4 (1.2)	5.8 (1.1)
Cover[2]	5.6 (1.2)	5.7 (1.0)	5.3 (1.0)	5.2 (1.2)
Hair quality[2]	5.3 (1.2)	5.1 (1.2)	5.3 (1.5)	4.8 (1.3)
Hair density[2]	5.2 (1.3)	5.2 (1.2)	5.7 (1.2)	5.3 (1.4)
Texture[2]	5.3 (1.4)	5.0 (1.4)	5.3 (1.0)	5.0 (1.1)
Overall Impression[2]	4.9 (1.1)	4.8 (1.0)	5.4 (1.0)	5.0 (1.3)

[1]Grading points from 1 (dark) to 10 (pale). [2]Grading points from 1 (poorest) to 10 (best).
No significant differences were found.

References

AOAC (1980) Official Methods of Analysis. 13th edn. Association of Official Analytical Chemists, Washington D.C.

Berge, G.M., Baeverfjord, G., Skrede, A. & Storebakken, T. 2004. Bacterial protein meal as protein source in diets for Atlantic salmon (*Salmo salar)* in saltwater. Aquaculture. In press.

SAS (1990) SAS User´s Guide: Statistics. Version 6. 4th edn., Cary, NC.

Øverland, M., Skrede, A. & Matre, T. (2001) Bacterial protein grown on natural gas as feed for pigs. Acta Agric. Scand., Sect. A, Animal Sci. 51: 97-106.

Roth, F.X. 1980. Micro-organisms as a source of protein for animal nutrition. Anim. Res. Dev. 12: 7-19.

Rouvinen, K., Archbold, S., Laffin, S. & Harri, M. 1999. Long-term effects of tryptophan on behavioural response and growing-furring performance in silver fox (*Vulpes vulpes*). Appl. Anim. Behaviour Sci. 63: 65-77.

Skrede, A., Berge, G.M., Storebakken, T., Herstad, O., Aarstad, K.G. & Sundstøl, F. (1998) Digestibility of bacterial protein grown on natural gas in mink, pigs, chicken and Atlantic salmon. Animal Feed Sci. Technol. 76: 103-116.

Skrede, A., Faaland Scøyen, H., Svihus, B. & Storebakken, T. 2003. The effect of bacterial protein grown on natural gas on growth performance and sensory quality of broiler chickens. Can. J. Anim. Sci. 83: 229.237.

Storebakken, T., Baeverfjord, G., Skrede, A., Olli, J.J. & Berge, G.M. 2004. Bacterial protein grown on natural gas in diets for Atlantic salmon, *Salmo salar*, in freshwater. Aquaculture. In press.

Stringer, D.A. 1982. Industrial development and evaluation of new protein sources: micro-organisms. Proc. Nutr. Soc. 41: 289-300.

SCIENTIFUR
ISSN 0105-2403
Vol. 28, No. 3

Proceedings of the VIII International Scientific Congress in Fur Animal Production

IV: Genetics and Reproduction

Edited by:

Dr. Bert Urlings
Prof. Dr. Berry Spruijt
Dr. Marko Ruis
Ing. Louise Boekhorst

IV – 1 RP

Stochastic simulation of breeding schemes to improve economic genetic merit in mink production

Bente Krogh Hansen and Peer Berg
Dept. of Animal Breeding and Genetics, DIAS, Box 50, DK-8830 Tjele
e-mail: BenteK.Hansen@agrsci.dk

Summary

In this study selection within farms was compared with selection across farms connected with an exchange of breeding animals following a circular pattern between farms.

We expected that a systematic exchange of animals in a group of farms would increase the total economic genetic merit. Genetic gain is influenced by several factors, among others population size, which can be increased by collaboration between several farms. The circular exchange of animals was analysed to test the effect of type, age and proportion of exchanged animals and the effect of the number of farms in a collaboration group. In all cases the breeding goal includes body weight and litter size, and the genetic gain is estimated using stochastic simulation. The total economic genetic merit per year is illustrated together with the rate of inbreeding during a period of 10 years.

Economic genetic merit varied from 7.6 to 8.5 DKK per mink per year. Increased weight genetic gain was obtained by exchange of yearling breeding animals. Furthermore, the annual increase of inbreeding was reduced from 0.86% per year in within farm selection to 0.42% per year with selection of animals across farms.

It is concluded that the economic genetic merit in a mink population can be improved by collaboration with other mink populations.

Introduction

In mink production each farm is a breeding unit, with its own breeding goal. Apart from selection among own stock some new breeding animals are bought based on auction or pelt show results. However these are not reliable indicators of genetic differences between farms. Most breeding units are small, which can lead to increased rate of inbreeding (Berg, 1997). In fur production high inbreeding tends to decrease reproduction (Nielsen & Berg, 1993; Nordrum, 1993; Berg, 1996), especially if the selection is on litter size in a small unit (Berg, 1997).

A group of fur producers with a common breeding goal could exchange breeding animals between farms to establish genetic links between farms. This increase of population size will consequently improve the genetic gain due to higher selection intensity, larger accuracy, and decreased rate of inbreeding.

In this project we used stochastic simulation to estimate the consequences of systematic exchange of animals within a group of farms on the total economic genetic merit. The economic genetic merit together with the genetic gain per year for litter size, body weight and pelt quality is presented in each scenario.

The aim of this paper is to test whether the economic genetic merit per year is higher when

- animals are selected within farms versus across farms
- males versus females are selected across farms
- larger proportions of animals are selected across farms
- more farms are included in a Breeder Group

Material and Methods

Statistical programmes

MINKSIM is a programme package for stochastic simulation specially applied to fur animal production. It is composed of a set of programmes developed as flexible tools for stochastic simulation of breeding schemes in farmed animals (e.g mink: Berg, 1997; sheep: Lauridsen, 1998, and cattle: Sørensen, 1999; Nielsen et al., 2001). The programme package MINKSIM simulates breeding work in a commercial farm and includes separate modules for each production period: -mating, -reproduction, -breeding value estimation and selection (Hansen & Berg, 2003).

The simulations of the data used in the analyses are based on the assumptions described in Table 1 and the scenarios we have chosen to illustrate alternative breeding schemes (see Collaboration pattern). Each scenario is repeated 25 times.

Table 1. Base values for some production parameters

Type of lines	Pure bred lines, registrations on all animals
Line size	200 dams + 40 sires
Mating	Random mating, 5 females per male
Percent of fertile dams	92 %
Mean values $_{(\pm std)}$ for traits	Litter size: 1st litter: 5.3 $_{\pm 2.3}$ 2nd litter: 5.6 $_{\pm 2.0}$ 3rd litter: 5.0 $_{\pm 2.5}$ Male weight: 2400 $_{\pm 250}$ Pelt quality: 3 $_{\pm 1}$
Selection principles	Truncation selection on an index: $I=0.07*ebv_{bw}+ 12*ebv_{ls}$, where ebv_{bw} and ebv_{ls} are estimated breeding values for body weight and litter size, respectively.
Simulation period	15 years, results from the last 10 years is used
Number of runs per year	25, except in the scenario (4) with different number of farms where 50 repetitions were used.

Simulation of animals

To produce a population that illustrates the situation on a commercial farm in a Breeder Group, each animal is simulated, with a corresponding breeding value and phenotypic observation. For each simulated animal genetic and phenotypic records are generated from predefined statistical distributions, taking the reduced variance due to Bulmer-effect and inbreeding into account (Bulmer, 1974). For details, see Sørensen, 1999; Sørensen et al. 1999.

Estimation of predicted breeding values

In the simulation program the prediction of breeding values is done once a year as in commercial mink farming. Breeding values for all animals from a Breeder Group are predicted simultaneously and the selection is based on these breeding values. Prediction of breeding values is carried out by univariate Animal Models using the programme package DMU (Jensen et al., 1997; Madsen & Jensen, 2000), corresponding to the model used in the Danish 'CFC-avl' Hansen et al. (1999), and including fixed effects of herd and year.

Genetic assumptions

When simulating breeding values including several traits, knowledge about genetic and environmental parameters for the traits simulated are needed. Genetic parameters known from earlier research (Hansen & Berg, 1997) and from literature in general (Berg, 1993a; Berg, 1993b) are used (Table 2). The habitability for litter size in each parity is adjusted according to the total phenotypic variance assuming the same genetic variation in all parities. The genetic correlation between 1., 2. and 3. litter is set to 1 and the genetic correlation between body weight and litter size to –0.30, and the genetic correlation between adult body weight and pelt quality to –0.04 (according to results of Lagerkvist et al., 1994).

Breeding goal

The aim is to maximize the economic index, which is based on a combination of the economic value of one unit of the studied traits. Skin length and litter size are both very important for the economic result of mink production and are therefore chosen as the

Table 2. Heritabilities (on the diagonal), genetic correlations (above) and phenotypic correlations (below the diagonal)

Trait	Litter_1	Litter_2	Litter_3	Body weight	Pelt quality
Litter_1	**0.08**	1	1	-0.30	-0.01
Litter_2	0.6	**0.11**	1	-0.30	-0.01
Litter_3	0.4	0.6	**0.07**	-0.30	-0.01
Body weight	0.09	0.09	0.09	**0.50**	-0.04
Pelt quality	-0.01	-0.01	-0.01	-0.10	**0.35**

first traits to be studied. Increasing skin length has a strong positive effect on skin price and increased litter size reduces production costs. Animals are ranked and selected due to this economic index.

The economic value of individual traits

Based on analyses of pelt prices of scanblack and scanbrown males in years 2000-2002 (Clausen, 2002) a mean value of 7 DKK per cm male pelt is estimated. As there is a high correlation between body weight in November and pelt size (Lohi & Hansen, 1989; Hansen & Lohi, 1990; Hansen et al., 1992), body weight at grading is here used as an estimate for skin size. 100 g extra body weight at pelting is calculated to yield an addition of 1 cm in pelt length (Møller, 2002). Applied to the above figures of pelt price per cm this gives an equation: 1 g extra body weight = 0.07 DKK extra price.

Litter size is recorded at the age of 14 days. According to Lagerkvist (1997) increased litter size will reduce the production costs with 12 SEK per skin when litter size increases from 6 to 7 at an assumed pelt price level of SEK 200 and feed price of 2 SEK per kg.

All three production traits, litter size, body size and pelt quality are simulated as continuous variables. Litter size is rounded to integers and set to zero if negative. Five percent of the females are randomly selected to be barren. Together with the distribution of litter size this results in approximately 10 % barren females in total. Consecutive records of litter size of the same female have a repeatability of 0.4 to 0.6, corresponding to a genetic correlation of 1. Five percent of both males and females are culled for reasons not correlated to the selection criterion (mortality or selection on other traits). All barren females are discarded. Variation in bodyweight for males and females is simulated to be similar, assuming that female weights are transformed to the scale of male weights.

Variation between farms is illustrated in the last year for each sub scenario (Table 4), and is used in relation to the economic genetic merit to compare the farms within a Breeder Group.

Rate of inbreeding. The inbreeding for each animal is estimated as the Wrights coefficient of inbreeding using the algorithm described by Meuwissen & Luo (1992). Rate of inbreeding in a sub scenario illustrates one of the consequences of the breeding scheme.

Collaboration pattern in a Breeder Group

To analyse the effect of collaboration four scenarios with sub scenarios are selected. Combinations are simulated with at least 25 replications. Within each replication breeding values and the realised observations for all animals are stored. From these data the average genetic merit, the average rate of inbreeding and the average genetic variation for animals born within the same year are calculated.

Scenario 1:
- Flow of animals within and across farms:
- a) Within farm selection, b) Selection across farm,
- c) Circular exchange of breeding animals between farms - within farm selection and exchange of a fixed proportion of animals.

Scenario 2:
- Type of breeding animals to be exchanged:
- a) Males or females, b)Yearling or adults

Scenario 3:
- Proportion of exchanged breeding animals: - 15, 30 or 50 %

Scenario 4:
- Size of the *Breeder Group:* 2, 3 or 5 farms in the group.

Statistical analysis of the collaboration effects

In each scenario different effects are studied by a univariate analysis. Simulation results from each year (se later) are analysed in a model considering the main effects and the corresponding interactions.

Scenario 1: $Y_{ij} = \mu + F_i + e_{ij}$

where Y_{ij} is the regression of the simulated trait on each year from each replication, e.g the annual economic genetic merit, μ is the general mean, F_i is the effect of flow of animals (i=1,2,3), e_{ij} the residual of the jth replication (j=1,…….25).

Scenarios 2 and 3:

$$Y_{klmn}=\mu+A_k+P_l+S_m+A_k*P_l+A_k*S_m+P_l*S_m+P_l*S_m*A_k+e_{klmn}$$

where Y_{klm} is the regression of the simulated trait on year from each replication, e.g the annual economic genetic merit, μ is the general mean, A_k is the effect of age of animals (k=1,2,3) males and females having two and three age groups, respectively, P_l is the effect of proportion of exchanged animals (l=15,30, 33/50) where adult males have a maximal proportion of 33%, S_m is the effect of sex of animals (m=1,2), A_k*P_l is the interaction between age and proportion, A_k*S_m is the interaction between age and sex, P_l*S_m is the interaction between

proportion and sex, e_{klmn} the residual of the nth replication (n=1,……25). As males was only accepted for breeding in two years, a maximum of 33 % of adult males could be exchanged.

Scenario 4: $Y_{op} = \mu + N_o + e_{op}$

where Y_{op} is the regression of the simulated trait on each year from each replication, e.g the economic genetic merit, μ is the general mean N_o is the effect of number of farms (i=2,3,5), e_{op} the residual of the pth replication (p=1,……50).

The comparison of scenarios is based on the total economic genetic merit (DKK) per year and compared with the sub scenario with 'Within farm selection'.

Results and Discussion

Scenario 1. The effect of different flow of animals across farms

Selection across farms results in a higher economic genetic merit, a lower rate of inbreeding and an increased genetic trend in body weight than selection within farms.

Response in Breeder Groups is compared in three sub scenarios. Two extremes: 'Within farm selection': in this case no animals are exchanged between farms meaning that the entire breeding stock each year is selected from own animals; 'Total exchange': is selection across farms. In the third situation 'Circular exchange' 15% of the yearling breeding stock and 5% of the adult breeding stock are purchased from one farm, and a similar proportion sold to a third farm in a circular pattern.

Economic genetic merit in Breeder Groups practising exchange of breeding animals is higher (8.37 and 8.48 DKK, respectively) than in the group with 'within farm selection' of animals (8.10 DKK), corresponding to a higher economic genetic gain of 3-5%.

Exchange of breeding stock, total or circular, reduces the variation between farms. Based on order statistics, the economic genetic merit per year on the best of the 3 farms in the group with within farm selection is 8,46 DKK, which means that in this case the best farm in the group is only as good as the average result for all farms with exchange of animals following a circular pattern.

The rate of inbreeding depends on the amount of exchanged breeding animals. Breeder Groups with within farm selection of animals have the highest rate of inbreeding and Breeder Groups with total exchange of breeding animals the lowest rate of inbreeding. The rate of inbreeding is reduced with approximately 50% when 20 percent of the breeding animals are exchanged and with 62% if all breeding animals are exchanged (Table 3).

Of the three traits studied only the genetic gain in body size is significantly influenced by the animal flow between farms. On average the genetic gain is 126 g per animal per year, being lowest in 'within farm selection' and 3% and 4% higher in the two other groups. Both in litter size and in pelt quality the genetic trend is negative and does not depend on the flow of breeding animals.

Table 3 Flow of breeding animals – selection within farm or across farms: total exchange or circular exchange The total economic genetic merit (DKK), rate of inbreeding, litter size (kits per litter), genetic gain in body weight (g) and genetic gain in pelt quality (percent change of a quality score).

	Selection			
	Within farms	Across farms		
	No exchange	Total exchange	Circular exchange	p-level
Economic genetic merit	$8.10_{\pm 0.05}$	$8.37_{\pm 0.05}$	$8.48_{\pm 0.07}$	***
Variation between farms[1)]	$0.18_{\pm 0.03}$	$0.01_{\pm 0.00}$	$0.03_{\pm 0.01}$	-
Rate of inbreeding	$0.0087_{\pm 0.0001}$	$0.0033_{\pm 0.0001}$	$0.0042_{\pm 0.0001}$	***
Genetic trend in: -litter size	$-0.058_{\pm 0.003}$	$-0.060_{\pm 0.003}$	$-0.051_{\pm 0.004}$	Ns
-body weight	$125.60_{\pm 0.50}$	$130.00_{\pm 0.69}$	$129.79_{\pm 0.83}$	***
-pelt quality	$-0.021_{\pm 0.003}$	$-0.015_{\pm 0.003}$	$-0.020_{\pm 0.004}$	Ns

[1)] *Variation of economic genetic merit between farms in the last year.*

Table 4. Total economic genetic merit, rate of inbreeding, and genetic gain in body weight, litter size and pelt quality related to type of animals, sex, age and proportion of exchanged animals. Average change per year (LSM) related to 'within farm selection'.

Type of collaboration and type of animals				Percent change per year in Breeder Groups with 'circular exchange' related to 'within farm selection' of animals					
collaboration	Sex	Age	Pro-portion	Total Economic	Variation between farms[1)]	In-breeding	Body weight	Litter size	Pelt quality
Within farm selection	-	-	-	8.09 DKK	$0.18_{\pm0.03}$	0.0086%	125.53g	-0.058 kit	-0.021point
circular	Male	yearling	15	$+1^{ns}$	$0.06_{\pm0.01}$	-51^{***}	$+2^{**}$	-10^{ns}	-10^{ns}
			30	$+4^{***}$	$0.03_{\pm0.01}$	-55^{***}	$+4^{***}$	-6^{ns}	-3^{ns}
			50	$+3^{**}$	$0.02_{\pm0.00}$	-61^{***}	$+4^{***}$	-10^{ns}	-7^{ns}
circular	Male	adult	15	-0.3^{ns}	$0.10_{\pm0.02}$	-44^{***}	0^{ns}	$+3^{ns}$	$+27^{ns}$
			30	-3^{**}	$0.04_{\pm0.01}$	-55^{***}	-2^{**}	-7^{ns}	$+16^{ns}$
			33	-4^{***}	$0.05_{\pm0.01}$	-56^{***}	-4^{***}	$+5^{ns}$	-22^{ns}
circular	Female	yearling	15	$+2^{*}$	$0.08_{\pm0.01}$	-47^{***}	$+2^{*}$	$+4^{ns}$	$+29^{ns}$
			30	$+3^{**}$	$0.05_{\pm0.01}$	-58^{***}	$+3^{***}$	-0.3^{ns}	$+14^{ns}$
			50	$+2^{*}$	$0.03_{\pm0.00}$	-61^{***}	$+3^{***}$	-4^{ns}	$+6^{ns}$
circular	Female	adult	15	-1^{ns}	$0.09_{\pm0.02}$	-44^{***}	0^{ns}	-13^{ns}	-6^{ns}
			30	-2^{ns}	$0.08_{\pm0.01}$	-56^{***}	0^{ns}	-14^{ns}	-12^{ns}
			50	-6^{***}	$0.03_{\pm0.01}$	-59^{***}	-5^{***}	-10^{ns}	-0.6^{ns}

$^{ns}p>0.05$; $^{*}p<0.05$; $^{**}p<0.01$; $^{***}p<0.001$
1) Variation of economic genetic merit between farms in the 15. year.

Scenarios 2 and 3. The effect of sex, age and proportion of exchanged animals

The results of different alternatives of 'circular change' are presented in Table 4 as the difference compared to the corresponding value if no breeding animals are exchanged. The main result is that it is preferable to exchange young animals as higher economic genetic merit and lower inbreeding can be expected than when exchanging adult breeding stock.

Economic genetic merit is influenced by age, proportion and up to some degree by sex of the exchanged animals.

Both in males and females, the exchange of young animals is more beneficial (Table 4) resulting in 2% to 4% higher economic genetic merit. The larger the proportion of adult males or females exchanged the smaller is the economic genetic merit per year.

Comparing exchange of young animals to adults the maximum difference in economic genetic gain in favour of young animals is 7 % and 5% in males and females, respectively.

The variation between farms is low in both alternative age and sex groups. Variation between farms decreases with increasing number of animals exchanged.

Generally there is no difference between the sexes in economic genetic merit per year. Thus it is the proportion of exchanged animals that determines the effect, and fewer males need to be exchanged to obtain the same change in response to selection.

Rate of inbreeding is increasing per year and is affected primarily by the proportion and slightly by the age of exchanged animals.

The rate of inbreeding in all Breeder Groups is significantly lower than if 'within farm selection' occurs (Table 4). The rate of inbreeding decreases with an increasing amount of exchanged breeding animals, despite the sex and age of the breeding animals.

Exchanging 15 percent of breeding animals will reduce the rate of inbreeding with 44% to 51%.

In Breeder Groups with 15 percent exchange of yearling males, the rate of inbreeding is lower than in the Breeder Groups with corresponding proportions of adult males or adult or young females.

In females there is no difference between the rates of inbreeding due to the age.

The genetic gain in body weight per year is significantly higher in groups exchanging young breeders (2% to 4%) compared to corresponding exchange of adult breeders (-2% to -5%) (Table 4). Already with 15 percent exchange of young breeding animals the genetic gain per year is higher than with a corresponding exchange of adult animals.

The genetic gain in litter size is decreasing in all cases and is not affected by factors included in the full model. There is no difference between any of the sub scenarios or compared to the Breeder Group with within farm selection of breeding animals.

The genetic gain in pelt quality varies between the different alternatives but no significant differences were found.

Scenario 4. Breeder Groups with different number of farms.

The advantage of more farms in Breeder Groups is the reduced rate of inbreeding. No difference was found in economic genetic merit, or in the three traits litter size, body weight or pelt quality. This indicates that increasing population size beyond 400 females is not advantageous.

Conclusion

Increased proportion of exchanged young animals increases the economic genetic merit.

Increased proportion of exchanged adult animals will decrease the economic genetic merit gradually.

The rate of inbreeding decreases with increasing amount of exchanged young animals and increasing number of farms in a Breeder Group.

Table 5. Number of farms in a Breeder Group with exchange of 30 % adult breeding males. Total economic genetic merit (DKK), rate of inbreeding, litter size (kits per litter), genetic gain in body weight (g) and genetic gain in pelt quality (percent change of a quality score). (50 repetitions)

Per year	No. of farms in a Breeder Group			
	2	3	5	p-level
Economic genetic merit (DKK)	$7.99_{\pm 0.05}$	$7.88_{\pm 0.04}$	$7.97_{\pm 0.03}$	ns
Variation between farms[1)]	$0.03_{\pm 0.01}$	$0.06_{\pm 0.01}$	$0.08_{\pm 0.01}$	-
Rate of inbreeding	$0.0055_{\pm 0.0001}$	$0.0041_{\pm 0.0001}$	$0.0041_{\pm 0.0001}$	***
Genetic trend in:				
- litter size	$-0.054_{\pm 0.004}$	$-0.062_{\pm 0.002}$	$-0.057_{\pm 0.002}$	ns
- body weight	$123.50_{\pm 0.54}$	$123.17_{\pm 0.41}$	$123.52_{\pm 0.40}$	ns
- pelt quality	$-0.019_{\pm 0.0031}$	$-0.017_{\pm 0.002}$	$-0.020_{\pm 0.002}$	ns

[1)] *Variation between farms in the last year in economic genetic merit*

References

Berg, P. 1993a. Variation between and within populations of mink. I. Weight and skin length Acta Agric. Scand., Sect. A. Animal. Sci. 43, 151-157.

Berg, P. 1993b. Present knowledge about heritability of different traits in mink. NJF-Forskermøde 29 april 1993. 10 pp.

Berg, P. 1996. The effect of inbreeding on reproduction and production traits in mink. In: Progress in Fur Animal Science, A. Frindt & M. Brzozowski eds. Applied Science Reports 27, 57-62

Berg, P. 1997. Realized inbreeding in simulated mink populations. NJF utredning / Rapport nr. 116. pp 235-242.

Bulmer, M.G. 1974. Linkage disequilibrium and genetic variability. Genet. Res. 23: 281-289.

Clausen, J. 2002. NJF-prisanalyser for salgssæsonen 2000/2001 og 2001/2002. http://www.cfc.dk/sw1378_fr_content.asp?StoreId=172258

Jensen, J., Mäntysaari, E.A., Madsen, P. and Thompson, R., 1997. Jour. Ind. Soc. Ag. Statistics 49, 215-236.

Hansen, B.K. & Berg P. 1997. Mink Kit Growth Performance in the suckling period. II. Estimates of Sources of Variation. Acta Agric. Scand., Sect. A. Animal. Sci. 47, 240-246.

Hansen, B.K. & Berg, P., 2003. Collaboration between mink farms improves response to selection. NJF's Subsektion for Fur Animals, Lillehammer, Norway, NJF Seminar 354, 8 pp.

Hansen, B.K., Berg, P. & Jensen, J., 1999. Genetic parameters for production traits in mink.

50th EAAP, Zurich, Switzerland, 23-26 August. Book of Abstracts No. 5, 23.

Hansen, B.K. & Lohi, O. 1990. Sammenhæng mellem minkhvalpens udvikling i dieperioden og i vækstsæsonen, samt korrelationer til pelsegenskaber. NJF Seminarium nr 185, 10 pp.

Hansen, B.K. , Lohi, O., P. Berg. 1992. Correlation between the development of mink kits in the lactation and growth periods, correlations to fur properties and heritability estimations. Norwegian Journal of Agricultural Sciences, Suppl. no.9 p. 87-93.

Lagerkvist, G., K. Johansson and N. Lundeheim 1994. Selection For Litter Size, Body Weight and Pelt Quality in Mink (*Mustela vison).* Correlated responses J. of Anim. Sci. 72 (5): 1126-1137.

Lagerkvist, G. 1997. Economic Profit from increased Litter Size, Body weight and Pelt Quality in Mink (Mustela vison), Acta. Agric Scand. Sect. A. Animal. Sci. 47:57-63

Lauridsen, J. 1998. Stochastic simulation of alternative breedign schemes for Danish meat type sheep. Ph.D Thesis. Pp 130.

Lohi, O. & Hansen, B.K. 1989. Heritabilitet af kropsvægt og kropslængde hos mink og størrelsesudvikling i relation til fødselstidspunkt og kuldstørrelse. NJF. Seminarium Nr. 170. 9 pp.

Madsen, P. & Jensen J. 2000. A User's Guide to DMU- A Package for Analysing Multivariate Mixed Models. Version 6, release 4. Danish Institute of Agricultural Sciences, Research Center Foulum, Box 50, 8830 Tjele, Denmark. 18 pp.

Møller S.H. 2002. Pelsningstidspunktets betydning for skindkvalitet IN: Damgaard & Hansen, 2002 Kvalitetsskind fra sunde mink, Intern rapport nr 163, 79-83.

Meuwissen, T.H.E. & Luo, Z. 1992. Computing inbreeding coefficients in large populations. Genet. Sel. Evol. 24:305-313.

Nielsen, U.L & Berg, P. 1993. Evaluering af indavlsforsøg med mink på forsøgsfarm Syd. NJF-utredning/rapport nr 92 NJF seminar nr. 239. p 53-58.

Nielsen, L.P., Sørensen, M.K., Berg, P. & Jørgensen, J.N. 2001. Alternative avlsstrategier for Rød Dansk Malkerace, DJF-rapport nr. 32. 99 pp.

Nordrum, N.M.V. Effekten af innavl på reproduksjonsegenskaber hos blårevtisper. NJF-utredning/rapport nr 92 NJF seminar nr. 239. p 59-69.

Sørensen, M.K. 1999. Stokastisk simulering af avlsplaner for malkekvæg, DJF-rapport nr 13. 183 pp.

Sørensen, M.K., Berg, P., Jensen, J. & Christensen, L.G., 1999. Stochastic simulation of breeding schemes for total merit in dairy cattle. GIFT Seminar on Gentic Improvement of Functional Traits in Cattle, Wageningen, The Netherlands, 7-9. November. Bulletin No. 23, 183-192.

IV – 2 RP

Inbreeding in a commercial fur animal breeding program

Kai-Rune Johannessen[1], Ejner Børsting[2] & Helen Kristiansen[1]
1) Norwegian Fur Breeders Association, Oslo, Norway, post@norpels.no
2) DK-4632 Bjæverskov, Denmark, ejner@borsting.dk

Abstract
A number of mink populations from 6 farms, which all uses the Norwegian field data recording system, 'Pelsdyrkontrollen', have been analysed for level of inbreeding and its possible effect on the reproduction results. Pedigree information is available for 51.900 mink over a period of 6 to 10 years, and 3.237 females have litter size recorded in 2003. The regression of litter size on inbreeding was –0,02678 ± 0,01564 (p=0,0869).
Calculations of average inbreeding coefficient on all whelps compared to the same calculations for those which are selected as new breeders shows that selection for reproduction seem to work against the general tendency to increase the inbreeding coefficient.
Approximately 150 Norwegian farmers use 'Pelsdyrkontrollen' for selection of new breeding animals. The module for selection based on live grading, which is used by approximately 1/3 of these farmers, has been enhanced with a tool that can help the farmer to maintain as high effective population size as possible on the farm. This makes it easier to control the rate of inbreeding.

Introduction
Inbreeding is the mating of animals that have ancestors in common, such that at a particular locus their progeny may be homozygous for an allele, which belonged to one ancestor (Cameron, 1997).
The most important consequence of elevated levels of inbreeding is the fact that inbred animals generally perform poorer than their non-inbred counterparts. Some of this poorer performance can be ascribed to the effects of negative recessive genes. More importantly, it seems that higher levels of inbreeding affect the fitness traits (reproduction and survival) the most and this is highly detrimental in any herd or breed.
The most used method of determining inbreeding is the inbreeding coefficient, introduced by Wright in 1921. It's called F and is defined as the correlation between genetic values of gametes.

The prediction of rate of inbreeding is often referred to as the simplified equation (Falconer, 1989)

$$\Delta F \approx \frac{1}{8N_m} + \frac{1}{8N_f}$$

where N_m and N_f are the numbers of breeding males and females in the population.

In fur animal farms the number of males is normally restricted compared to the females, thus the effect of population size is determined much by the number of breeding males. This will reduce the effective population size and increase the rate of inbreeding (ΔF).
In a closed population the rate of inbreeding will increase regardless of which selection and mating system is chosen. That means that it is important to know more about an individual than it's grandparents, to be able to reduce or control the rate of inbreeding.
Most breeding programs in principal result in a reduction in the genetic variation caused by the estimating model of breeding values, as the programs have tendencies to pick animals from the same families. In small populations the risk of fixation of genes and elimination of genes by random drift is higher than in larger populations and will increase the risk of building up homozygous loci, i.e. inevitably reduce the genetic variation. On the other hand the possible negative effect of inbreeding on the reproductive performance will tend to have the opposite effect i.e. the inbred animals, which performs poorer, will get lower indices and will contribute to fewer new breeding animals in the next generation.
The normal mink farm in Norway is rather small, with an average of approximately 500 breeding females, often divided into two or three "purebred" colour types. Many of the farms are situated at long distances from each other making the exchange of animals between farms difficult. The danger of contagious diseases, such as plasmacytoses also

makes the exchange of animals complicated. Most of the farms operate their own breeding programme, thus a mink farm may often be looked upon as a closed population with a rather small effective population size.
This investigation has been performed to make an overview of the general situation of a typical Norwegian mink farm and give the breeders a tool to monitor their own situation and have some practical control on the rate of inbreeding in their population.

Material and methods

Data from 6 different mink farms in the central database of the Norwegian field control system ‘Pelsdyrkontrollen’ have been used in this investigation.
The distribution of the pedigree information in the material is shown in table 1.
The data contains pedigree of approximately 10 years, which gives almost 10 generations of mink. The database was not originally designed for calculations and monitoring of inbreeding, thus there was made an extraction of the pedigrees and the data was transformed to be handled by Microsoft® Excel and analysed by SAS® Stat. Software.
The rate of inbreeding was calculated by Proc.Inbred of SAS® Stat. as the inbreeding coefficient designed by Wright. The calculations were made within each farm.
The effect of inbreeding on the reproductive performance of the minks was investigated by analysing the possible effect of inbreeding on litter size. Both litter size at birth and litter size at 3 weeks were used in the analyses.
However the initial analyses showed that analysing on numbers of kits at 3 weeks gives more consistency in the material. This is to be expected, as many breeders are more likely to make an accurate count of live kits at 3 weeks, than they do at birth. A total of 3237 females with litter size > 0 at 3 weeks in 2003 were included in the calculations.
Data were analysed with a linear model with farm, year of birth (female age) and mink type as fixed effects. The regression of the inbreeding coefficient of the female on her litter size in 2003 was calculated.

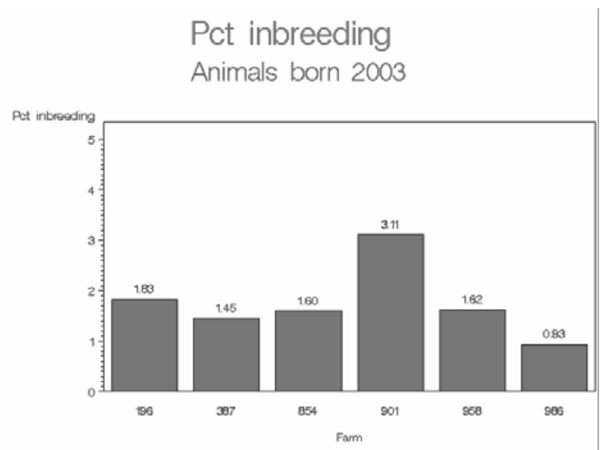
Table 1: Distribution of animals in the pedigree on year of birth and farm

No.	Year											All
	1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	animals
Farm												
196	1	6	31	145	228	334	749	890	881	5185	4282	12732
387		2	13	20	63	79	126	139	203	910	1104	2659
854			17	87	134	186	405	351	385	2679	2444	6688
901	1	21	46	102	166	168	359	484	599	3609	3841	9396
958	1	6	24	86	142	189	435	580	486	3629	3346	8924
986		6	16	95	182	337	726	892	798	4517	3932	11501
All	3	41	147	535	915	1293	2800	3336	3352	20529	18949	51900

Results

The average degrees of inbreeding in the farms are shown in fig.1.

Figure 1: Average inbreeding per farm of animals born in 2003



The general impression is that there is no alarmingly high degree of inbreeding in the farms, on the other hand there are some differences between the farms. It is essential to remark that the inbreeding coefficients in the material are results of the pedigrees as they look at this moment, and does not include any earlier inbreeding in the animals forming the “base population”.

The effect of inbreeding on reproduction in the total material of the 6 farms was calculated with litter size at birth and at 3 weeks, shown in table 2.

Tabel 2. Regression of litter size on degree of inbreeding

	N	b	st.d.	p
Litter size at birth	3407	- 0,02533	0,01720	0,1410
Litter size at 3 weeks	3237	- 0,02678	0,01564	0,0869

The results are in good accordance with similar results from other publications on fur animals (Berg, 1996; Nordrum, 1993) and similar data from other farm animals (Pirchner, 1983).

The effect of inbreeding on reproduction (litter size at 3 weeks) can also be described by the effect of the actual selection. From data for 2002 we calculated the average inbreeding coefficient for all whelps and the average of those who were selected as breeders. In all 6 farms the inbreeding coefficient of the whelps selected as breeders was lower than the total whelp population in the same farm, as shown in table 3. This indicates that even though the indices might give an effect of inbreeding, in this material it seems that the negative effect of inbreeding on reproduction works in contradiction to the tendency from the indices to pick animals that are from the same family.

Table 3. Average inbreeding for all whelps and for the new breeding animals chosen amongst the whelps

Farm	All whelps	Selected breeders
196	1,4	1,0
387	1,9	1,6
854	1,1	0,8
901	3,1	2,5
958	1,0	0,9
986	1,5	1,0
Average	1,67	1,17

Discussion

The farms included in this investigation have approximately 200 to 1000 breeding females each thus giving a good picture of the mink farms in Norway. The results shows a clear effect from inbreeding on reproduction traits. The level of influence from inbreeding on litter size at 3 weeks is in accordance with similar results in other reports on inbreeding. The level of inbreeding was not high in the material, and as far as one can tell the rate of increase of inbreeding is not high. The effect of low rates of inbreeding can often be eliminated by selection. As an example 12 generations of breeding with a rate of 1% inbreeding, would create an inbreeding of 12%. The negative effects of this, however, will normally be eliminated over the same time by means of selection. 12 % inbreeding is the same as one generation of half sib matings, but this of course will have much greater effect, as it will not be subject to selection along the way.

The evidence of effects on reproduction from inbreeding in this material, and similar but mostly smaller effects on other production traits (Berg, 1996), still makes it relevant for commercial mink breeders to pay some attention to the effect of inbreeding in their breeding program.

Possible means of controlling the rate of inbreeding are (Cameron, 1997)

- Selection on biased predicted breeding values (increased heritability will reduce the tendency to select related animals)
- Breeding values corrected for genetic relations
- Control and monitoring of the family structure

Farmers using 'Pelsdyrkontrollen' are offered an overview of the level of inbreeding in their population and the effect of it on the reproduction. A new Excel® based service, has recently been introduced and made available on the Internet without extra cost for the members of 'Pelsdyrkontrollen'. An extract of the database is transformed to an Excel-file and located at a given Internet-address from where it may be downloaded to the farmers own PC. By a few mouse-clicks the Excel sheet can produce the statistics shown in table 4. This gives the actual level of inbreeding and the development over time.

Another statistic, which can be obtained by a new mouse-click is shown in table 5. Here the breeding animals are grouped according to type, year of birth and three categories of inbreeding. The results gives number of females in each group and average litter size at 3 weeks per group.

Table 4: Actual report on inbreeding distributed on mink colour type and year of birth. The numbers of animals of each type in the farm must be a part of the evaluation of the situation.

Average inbreeding	Year of birth							
Mink colour type	1997	1998	1999	2000	2001	2002	2003	Totalt
100	1,6	3,3	3,6	3,4	3,5	3,8	4,0	3,8
109		0,0	0,0	1,4	3,7	1,7	3,4	3,2
200		0,0	1,0	1,7	0,7	2,5	3,4	2,8
210	0,0	0,0	0,9	0,5	0,1	0,1	1,3	0,6
221	0,0	0,0	2,4	1,6	0,9	1,0	2,5	1,9
222		0,9	0,4	1,1	1,5	1,1	2,3	1,8
223			0,6	1,0	0,9	1,6	2,3	2,0
224				0,6	0,4	1,8	2,7	2,5
421		0,0	1,4	1,7	2,2	1,2	1,8	1,7
500	0,0	0,0	1,1	2,0	3,0	2,3	3,4	2,8
Total	1,0	1,1	1,6	2,1	2,4	2,5	3,1	2,8

Table 5: Reproduction performance (litter size at 3 weeks) of scanblack females grouped in 3 different categories of inbreeding and in year of birth. Numbers of females in the groups is to be considered.

			Inbreeding			
Type	Year	Data	**a=0-5**	**b=6-15**	**c=16-**	Total
100 (scanblack)	1998	Number of females	15	9		24
		Average litter size		**5,0**		5,0
	1999	Number of females	45	11		56
		Average litter size	**5,5**	**3,5**		5,2
	2000	Number of females	149	44	2	195
		Average litter size	**6,4**	**6,7**		6,4
	2001	Number of females	135	65	4	204
		Average litter size	**6,6**	**6,4**	**4,0**	6,5
	2002	Number of females	112	53	1	166
		Average litter size	**5,8**	**5,5**		5,8
Numbers of females			472	186	7	665

These statistics may be used by the breeder to decide if there are any problems regarding inbreeding in his farm, and aid in the decisions regarding the necessity of import or other measurements to avoid negative effects of inbreeding.
The Excel-tool for selection, based on fertility indices and live grading results, also supplied by 'Pelsdyrkontrollen', has now been enhanced by a facility to control the numbers of animals selected per sire. The consequences of the restriction-choices for family structure made by the breeder are shown immediately and the breeder may decide to accept the results or go back and recalculate with other restrictions on family structure. In this way the breeder may to a certain degree control and reduce the rate of inbreeding and at the same time know how much it costs in loss of progress in the production traits.

Conclusions and remarks

This investigation was meant to be a practical way of using the field data from the central database in 'Pelsdyrkontrollen' to investigate the situation regarding inbreeding in Norwegian mink farms and the possible effect of inbreeding on reproduction. Furthermore the goal was to give the farmers the option to "scan" their populations of minks, find the level of inbreeding and offer a tool to monitor the rate of inbreeding. At the same time evaluate the costs of the reduced rate of inbreeding on the reduced progress of the production traits.
The Norwegian members of 'Pelsdyrkontrollen' will be offered these options from the 2004 production season. The system will be available through the Internett, in accordance with the system for live grading launched in 2000 based on the use of Microsoft® Excel.

References

Berg, P. 1996. The effect of inbreeding on reproduction and production traits in mink. In Frindt, A. & Brzozowski, M. (eds.) proceedings from the VI'th International Scientific Congress in Fur Animal Production. Applied Science Reports, Progress in Fur Animal Science, Suppl. 27: 57-62

Cameron, N.D. 1997. Selection Indices and Prediction of Genetic Merit in Animal Breeding. CAB International. ISBN: 0851991696.

Falconer, D.S. 1989. Introduction to Quantitative Genetics, Third Edt. Longman. 438 pp

Nordrum, N.M.V. 1993. Genetic and endocrinological factors influencing reproduction in blue foxes. Agricultural University of Norway. Dr. scient. Theses. 1993, 75 pp.

Pirchner, F. 1983. Population Genetics in Animal Breeding. Sec. Edt. Plenum Press. 414 pp.

SAS Institute Inc., SAS/ Stat. ® Software Changes and Enhancements, through Release 6.11, Cary, NC: SAS Institute Inc., 1996. 1044 pp.

IV – 3 RP

Genetics of litter size, age at first insemination and animal size in blue fox (*Alopex lagopus*)

Jussi Peura[1], Ismo Strandén[1] and Kerstin Smeds[2]
1)MTT Agrifood Research Finland, Animal Production Research, 31600 Jokioinen,
Jussi.Peura@mtt.fi *2)Finnish Fur Breeders Association, 01601 Vantaa*

Abstract

Skin size of blue fox has increased considerably in Finland during the last decade. This may have lead to decreased fertility through unfavourable genetic correlation. The average number of pups per mated females has slightly decreased after the mid-90's. The objective of this study was to estimate the genetic parameters of the first three litter sizes, female age at first insemination and animal size using REML with single and multitrait animal models. The data was obtained from the Finnish Fur Breeders' Association. In the single trait analysis data and pedigree had 30268 and 44297 animals in litter size, 46295 and 62035 animals in age at first insemination and 68108 and 78775 animals in animal size, respectively. Multitrait analysis had 9126, 5115, 2525, 15381 and 23574 observations in 1st, 2nd and 3rd litter size, age at first insemination and animal size, respectively. Pedigree had 32356 animals in the multitrait analysis. Heritability estimates were 0.08, 0.08 and 0.07 for first, second and third litter size, respectively. Heritability estimates in single and multitrait analysis were 0.16 and 0.18 for age at first insemination and 0.24 and 0.25 for animal size, respectively. The genetic correlations between animal size and age at first insemination and first, second and third litter size were -0.20, -0.40, -0.40 and -0.23, respectively. Genetic correlations between first and second litter size were 0.62, between first and third 0.51, and between second and third 0.60. This study supports the conclusion that there is an antagonistic genetic correlation between fertility and animal size.

Introduction

During the last decade, one of the main goals in Finnish blue fox breeding has been to increase animal size. The average skin size has considerably increased during the last $10-15$ years. While the skin size has increased, the average number of pups per mated female has slightly decreased after mid-90's. Because few studies have been made about the genetic correlation between animal size and fertility of blue foxes, consequences of selection on skin size to fertility is unclear. However, Lagerkvist et al., 1994, found low antagonistic genetic correlation between fertility and animal size. The objective of this study was to estimate genetic parameters between litter size, age of female at first insemination and animal size. The main objective was to study if there is an antagonistic correlation between fertility and animal size. The secondary objective was to study how genetic increase in animal size affects the sexual maturity of young females.

Material and Methods

Data was obtained from the Finnish Fur Breeders' Association. The analyzed data set was a subset having 18 farms with observations from years 1989-2001. The farms were known to have breeding cooperation. In the data only purebred blue foxes were accepted.

The studied fertility traits were the first three litter sizes (LS) and females age at first insemination (AFI). Litter size observations from females with $1-20$ pups after $2-3$ weeks from whelping were accepted. However, litter size observations from females mated with more than one male per breeding season were excluded. Observations from litter sizes were excluded, if an observation from animal size was missing. Also observations from later (second and third) litter sizes were excluded if observation from an earlier litter size was missing. AFI was defined as number of days between date of birth and first recorded mating. Observations under 274 and over 367 days were assumed to be incorrect and were therefore excluded. The animal size (AS) was a subjective grading measurement made by the farmer. In Finland, the grading scale for animal size goes from 1 to 5 so that 1 is smallest and 5 is biggest. The grading is done so that the average size is approximately 3 in the farm each year. In Finland, grading is done to young animals mainly in October, just before the pelting season.

(Co)variance components were estimated both in single trait and multitrait animal models using the DMU program (Madsen & Jensen 2000) that relies

on restricted maximum likelihood (REML) method in variance component estimation. In the single trait analysis variance components of 1st LS, AFI and AS were estimated by model:

$$\mathbf{y} = \mathbf{Xb} + \mathbf{Wc} + \mathbf{Za} + \mathbf{e} \quad (1)$$

where **y** is vector of observations in 1st LS, AFI or AS and **b** is a vector of fixed effects, and **c**, **a** and **e** are vectors of random litter, animal and residual effects, respectively. **X**, **W** and **Z** are the incidence matrices for fixed, litter and animal effects, respectively. Model (2) was used in multitrait analysis.

$$\begin{bmatrix}\mathbf{y}_1\\\mathbf{y}_2\\\mathbf{y}_3\\\mathbf{y}_4\\\mathbf{y}_5\end{bmatrix}=\begin{bmatrix}\mathbf{X}_1&\mathbf{0}&\mathbf{0}&\mathbf{0}&\mathbf{0}\\\mathbf{0}&\mathbf{X}_2&\mathbf{0}&\mathbf{0}&\mathbf{0}\\\mathbf{0}&\mathbf{0}&\mathbf{X}_3&\mathbf{0}&\mathbf{0}\\\mathbf{0}&\mathbf{0}&\mathbf{0}&\mathbf{X}_4&\mathbf{0}\\\mathbf{0}&\mathbf{0}&\mathbf{0}&\mathbf{0}&\mathbf{X}_5\end{bmatrix}\begin{bmatrix}\mathbf{b}_1\\\mathbf{b}_2\\\mathbf{b}_3\\\mathbf{b}_4\\\mathbf{b}_5\end{bmatrix}+\begin{bmatrix}\mathbf{W}_1&\mathbf{0}&\mathbf{0}&\mathbf{0}&\mathbf{0}\\\mathbf{0}&\mathbf{W}_2&\mathbf{0}&\mathbf{0}&\mathbf{0}\\\mathbf{0}&\mathbf{0}&\mathbf{W}_3&\mathbf{0}&\mathbf{0}\\\mathbf{0}&\mathbf{0}&\mathbf{0}&\mathbf{W}_4&\mathbf{0}\\\mathbf{0}&\mathbf{0}&\mathbf{0}&\mathbf{0}&\mathbf{W}_5\end{bmatrix}\begin{bmatrix}\mathbf{c}_1\\\mathbf{c}_2\\\mathbf{c}_3\\\mathbf{c}_4\\\mathbf{c}_5\end{bmatrix}+\begin{bmatrix}\mathbf{Z}_1&\mathbf{0}&\mathbf{0}&\mathbf{0}&\mathbf{0}\\\mathbf{0}&\mathbf{Z}_2&\mathbf{0}&\mathbf{0}&\mathbf{0}\\\mathbf{0}&\mathbf{0}&\mathbf{Z}_3&\mathbf{0}&\mathbf{0}\\\mathbf{0}&\mathbf{0}&\mathbf{0}&\mathbf{Z}_4&\mathbf{0}\\\mathbf{0}&\mathbf{0}&\mathbf{0}&\mathbf{0}&\mathbf{Z}_5\end{bmatrix}\begin{bmatrix}\mathbf{a}_1\\\mathbf{a}_2\\\mathbf{a}_3\\\mathbf{a}_4\\\mathbf{a}_5\end{bmatrix}+\begin{bmatrix}\mathbf{e}_1\\\mathbf{e}_2\\\mathbf{e}_3\\\mathbf{e}_4\\\mathbf{e}_5\end{bmatrix} \quad (2)$$

where the trait numbers 1 -5 correspond to AS, AFI and 1st,2nd and 3rd LS, respectively. Fixed and random effects were the same in both single and multitrait analysis. Fixed effects for each trait are presented in table 1. Covariance matrices of random effects in single and multitrait models were assumed to be:

$$\mathrm{var}\begin{bmatrix}\mathbf{a}_s\\\mathbf{c}_s\\\mathbf{e}_s\end{bmatrix}=\begin{bmatrix}\sigma_a^2\mathbf{A}&\mathbf{0}&\mathbf{0}\\\mathbf{0}&\sigma_c^2\mathbf{I}&\mathbf{0}\\\mathbf{0}&\mathbf{0}&\sigma_e^2\mathbf{I}\end{bmatrix}$$

and

$$\mathrm{var}\begin{bmatrix}\mathbf{a}_m\\\mathbf{c}_m\\\mathbf{e}_m\end{bmatrix}=\begin{bmatrix}\mathbf{G}_0\otimes\mathbf{A}&\mathbf{0}&\mathbf{0}\\\mathbf{0}&\mathbf{C}_0\otimes\mathbf{I}&\mathbf{0}\\\mathbf{0}&\mathbf{0}&\mathbf{R}_0\otimes\mathbf{I}\end{bmatrix}$$

respectively, where s and m correspond with single and multitrait analysis, respectively, and σ_a^2, σ_c^2 and σ_e^2 are additive genetic, litter environment and residual variance, respectively. **A**, **I**, $\mathbf{G}_0$, $\mathbf{C}_0$ and $\mathbf{R}_0$ are numerator relationship matrix, identity matrix, 5×5 additive genetic covariance matrix, 5×5 covariance matrix for litter environmental effect and 5×5 covariance matrix for residual effects, respectively. Heritability and proportion of litter variation were calculated as

$h^2 = \sigma_a^2/(\sigma_a^2+\sigma_c^2+\sigma_e^2)$ and

$c^2 = \sigma_c^2/(\sigma_a^2+\sigma_c^2+\sigma_e^2)$, respectively.

Results and Discussion

Mean, standard deviation (s.d), coefficient of variation (CV), minimum and maximum in 1st, 2nd and 3rd LS, AFI and AS for the analyzed data are presented in table 2.

The average of 1st LS is about 2.5 pups smaller than in 2nd and 3rd LS. The standard deviations were similar in 1st, 2nd and 3rd LS. Nevertheless, the coefficient of variation in 1st LS is slightly larger than in 2nd and 3rd LS due to smaller mean in 1st LS. The difference in CV between 1st LS and later LS's is probably caused by strong selection between 1st and 2nd LS. The average of AFI is 319.98 days, which is about 10.7 months. The standard deviation of AFI is quite low (10.84) which causes considerably lower coefficient of variation (0.03) than in other traits studied $(0.18-0.49)$. The mean of AS was 3.99, showing, that above average values are given more often than desired.

Table 1. Fixed effects in single and multitrait analysis for 1st, 2nd and 3rd litter sizes (LS), age at first insemination (AFI) and animal size (AS).

	LS				
	1st	2nd	3rd	AFI	AS
farm-year	X	X	X	X	X
time of birth (for animal)[1)]	X			X	X
mating method[2)]	X	X	X		
number of matings[3)]	X	X	X		
age of dam[4)]					X
sex[5)]					X

1)4 classes (104-129, 130-144, 145-160 and 161-180 days from the beginning of the year)
2)2 classes (natural or artificial insemination)
3)2 classes (1 or >1 matings/season)
4)3 classes (1, 2 or 3-15 years old)
5)3 classes (male, female or pup)

Table 2. Mean, standard deviation (s.d), coefficient of variation (CV), minimum and maximum of 1st, 2nd and 3rd litter size (LS), age at first insemination (AFI) and animal size (AS).

trait		mean	s.d	CV	Minimum	Maximum
LS[1)]	1st	6.14	3.01	0.49	1	18
	2nd	8.68	3.38	0.39	1	19
	3rd	8.72	3.25	0.37	1	17
AFI[2)]		319.98	10.84	0.03	271	361
AS[3)]		3.99	0.72	0.18	1	5

1) number of pups 3 weeks after whelping, 2)days, 3) size points

Table 3. Animals in data and in pedigree, phenotypic variances (σ_P^2), litter environmental effect (c^2) and standard errors (s.e) and heritabilities (h^2) and their standard errors (s.e) in 1st litter size (LS), age at first insemination (AFI) and animal size (AS) in single trait analysis.

trait	Animals in data	Animals in pedigree	σ_P^2	$c^2 \pm s.e$	$h^2 \pm s.e$
LS	30 268	44 297	9.20	0.03± 0.01	0.08±0.01
AFI	46 295	62 035	96.97	0.26±0.01	0.16±0.01
AS	68 108	78 775	0.67	0.10±0.00	0.24±0.01

Heritabilities and proportion of litter variation in single trait analysis are in table 3. The heritability estimate of 0.08 for litter size was low, which agrees with Valberg Nordrum, 1993, and Nikula, 2000. In the literature, AFI has not been estimated for blue foxes. However, in pigs and dairy cattle the subject is widely studied. Raheja et al., 1989, had slightly smaller estimate of heritability (0.11) for heifers than in the present study for blue foxes (table 3). On the other hand Hanenberg et al., 2001, had higher estimates of heritability for gilts (0.32). In our study, the litter variation in AFI was 0.26, which is considerably larger than the heritability (0.16). This is probably due to large effects of dams nursing ability, competition between pups within litter and the location of the litter in the shed. When the litter effect was excluded, estimate of heritability was over 0.40.

Heritability of AS was smaller than in Kenttämies & Smeds, 2002. Wierzbicki, 2000, had higher heritability when data was not transformed. After probit transformation of data Wierzbicki, 2000, had heritabilities similar to the present study. However, the studies are not entirely comparable, because Wierzbicki, 2000, had no litter environment as a random effect in the model. In the present study, litter environment was 0.10, so it accounts for quite a large amount of variation in AS.

In multitrait analysis, number of observations was largest in AS whereas 3rd LS had smallest amount of observations (table 4). However, every trait pair had always over 2400 observations. The pedigree had 32356 animals in multitrait analysis. The heritabilities in multitrait analysis and genetic and phenotypic correlations between 1st, 2nd and 3rd LS, AFI and AS are in table 5. Heritabilities in 1st LS (0.08), AFI (0.18) and AS (0.25) were close to those in the single trait analysis. Heritabilities in 2nd and 3rd LS were close to 1st LS. Standard errors were slightly higher in multitrait analysis than in single trait analysis, which was probably due to smaller amount of observations per trait.

The genetic correlations between AFI and 1st, 2nd and 3rd LS were 0.26, 0.34 and 0.26, respectively. Thus genetically early sexually maturing animals had less pups three weeks after whelping than later maturing animals.

Genetic correlations between AS and 1st, 2nd and 3rd LS were -0.40, -0.40 and -0.23, respectively. Despite the smaller genetic correlation between AS and 3rd LS than between AS and 1st and 2nd LS, and despite standard error increasing along with parity number, the genetic correlation between AS and first three LS is clearly antagonistic.

The genetic correlation between AS and AFI was -0.20. Because grading is done in October when young animals are still growing, the shape of growth curve has a big impact on the grading size of blue fox. It seems, that the selection based on the grading size, increases the growth rate, which again via moderate genetic correlation makes animal mature sexually earlier.

The genetic correlation between 1st and 2nd LS was 0.62 between 1st and 3rd LS 0.51 and 2nd and 3rd LS 0.60. Correlations were high but still they supported the conclusion that 1st, 2nd and 3rd LS are at least partly different traits.

Table 4. Number of animals in each trait and trait pairs in multitrait analysis of 1st, 2nd and 3rd litter size (LS), age at first insemination (AFI) and animal size (AS).

		LS				
		1st	2nd	3rd	AFI	AS
LS	1st	9126				
	2nd	5115	5115			
	3rd	2525	2525	2525		
AFI		9105	5106	2523	15 381	
AS		8919	4969	2423	13 415	23 574

Table 5. Heritabilities and their standard errors (diagonal), genetic correlations and their standard errors (upper triangle) and phenotypic correlations (lower triangle) in 1st, 2nd and 3rd litter size (LS), age at first insemination (AFI) and animal size (AS) in multitrait analysis.

		LS				
		1st	2nd	3rd	AFI	AS
LS	1st	0.08±0.02	0.62±0.17	0.51±0.24	0.26±0.10	-0.40±0.09
	2nd	0.18	0.08±0.03	0.60±0.27	0.34±0.13	-0.40±0.12
	3rd	0.16	0.23	0.07±0.04	0.26±0.18	-0.23±0.16
AFI		0.08	0.04	0.00	0.18±0.02	-0.20±0.06
AS		-0.07	-0.07	-0.01	-0.02	0.25±0.02

Conclusion

Heritability of litter size was estimated to be low. The heritability of female age at first insemination was higher than in litter size. Large effect of the random litter effect in age at first insemination indicates that maternal effects may be considerable. In the future, it might be reasonable to estimate the genetic parameters by including maternal effects in the model. The heritability of animal size was moderate and lower than in previous studies made for Finnish blue foxes. Moreover, the moderate estimate of heritability in animal size cannot totally explain significant increase of skin size in the Finnish blue fox population during the last decade.

If the antagonistic genetic correlations between animal size and 1st, 2nd and 3rd litter size are not accounted, genetic increase in animal size will decrease the average litter size. However, because of the low heritability of litter size, the phenotypic impact may be quite small. The negative genetic correlation between animal size and age at first insemination indicates, that genetic increase in animal size will decrease age at first insemination. In the future it would be reasonable to study how different components of growth (fat, protein, water) and the shape of growth curve relate to the age at first insemination. This is interesting, because there was positive genetic correlation between age at first insemination and first three litter sizes. If large amount of breeding efforts is put to increase animal size, it will decrease litter size. Moreover, the litter size will decrease also indirectly via decrease in age at first insemination. It supports the conclusion that it might be reasonable to include age at first insemination in multitrait breeding value evaluation to support improvement in traits with economical importance.

References

Hanenberg, E., H., A., T., Knol, E., F. & Merks, J., W., M. 2001. Estimates of genetic parameters for reproduction traits at different parities in Dutch Landrace pigs. Livestock Production Science, 69(2): 179-186.

Kenttämies, H. & Smeds, K. 2002. Correlated responses in litter result, body size, fur quality and color clarity in blue foxes (*Alopex lagopus*) selected for confident behavior. 7 th World Conferess on Genetics Applied to Livestock Production, August 19-23, 2002, Montpellier, France.

Lagerqvist, G, Johansson, K. & Lundeheim, M. 1994. Selection for litter size, body weight, and pelt quality in mink (*Mustela vison*): correlated responses. Journal of Animal Science 72: 1126-1137.

Madsen, P. & Jensen, J. 2000. A user’s quide to DMU, a packade for analyzing multivariate mixed models, Danish Institute of Agricultural Sciences (DIAS). Tjele, Denmark. Mimeo 22 s.

Nikula, S. 2000. Kettujen luonteen periytyvyys ja yhteys hedelmällisyysominaisuuksiin. Pro Gradu –työ. Helsingin Yliopiston kotieläintieteen laitoksen julkaisuja 48. 31 s.

Raheja, K.L., Burnside, E.B. & Schaeffer, L.R. 1989 Heifer fertility and its relationship with cow fertility and production traits in holstein dairy cattle. Journal of Dairy Science 72: 2665-2669.

Valberg Nordrum, N. 1993 Genetic and endocrinological factors influencing reproduction in blue foxes. Agricultural University of Norway. Doctor Scientarum Theses 1993:7.

Wierzbicki, H. 2000. Additive genetic and error variance components for conformation and coat traits in arctic fox *Alopex lagopus* (L.). Scientifur 24(3): 217-222.

IV – 6 RP

Diapause, implantation and placentation in the mink: A critical role for embryonic signaling

Joëlle Desmarais, Flavia L. Lopes, Vilceu Bordignon and Bruce D. Murphy***
Centre de recherche en reproduction animale, Faculté de médecine vétérinaire, Université de Montréal, St-Hyacinthe QC Canada, J2S7C6
**Present address: Department of Animal Science, McGill University, Ste-Anne-de-Bellevue, QC Canada, H9X3V9*
***Corresponding author, email: bruce.d.murphy@umontreal.ca*
This study was supported by NSERC of Canada Discovery Grant 137103 to B.D. Murphy

Abstract
During the first six days following mating and ovulation, the mink embryo follows the usual mammalian pattern of development to the blastocyst stage. These embryos then undergo a period of obligate developmental arrest, known as diapause. We have studied the mechanisms regulating the sequence of events between the escape from diapause to the postimplantation invasion of the uterus. We have demonstrated marked increases in embryo diameter within 24 h, and in DNA and protein synthesis beginning as early as 48 h after the reinitiation of development. Culture of cells harvested from embryos at intervals demonstrated that the trophoblast proliferated more readily during the early reactivation phase, while the inner cell mass proliferated later. The signal for trophoblast proliferation was fibroblast growth factor-4 (FGF4) presumed to be produced by the inner cell mass, and acting on its cognate receptors in the trophoblast. Embryos reached approximately 2.0 mm in diameter prior to implantation into the uterus, some 11-12 days after reactivation. During reactivation, the blastocyst produces prostaglandins, particularly PGE_2, which then acts on uterine receptors of the EP-2 and EP-4 subtypes. The vascular endothelial growth factor (VEGF), a promoter of angiogenesis, is strongly expressed by the trophoblast cells of the implanting embryo, and transcription of the VEGF gene was induced by PGE_2 and PGD. The embryo is necessary for the local expression of both known forms of the VEGF receptor associated with the early stages of vascularization of the placenta. Our investigations indicate that, following the escape of the mink embryo from its arrested state, cascade of embryonic signals promote trophoblast development, blastocyst invasion, and vascularization of the placenta.

Introduction
Mink gestation is characterized by a discontinuity in development of the embryo, occurring at the blastocyst stage. This condition, known as embryonic diapause, evolved as a strategy for the timing and syncronization of parturition at time favorable to the survival of offspring (Thom, Johnson *et al.* 2004). The developmental trajectory of the mink embryo was described by Hanssen in his comprehensive investigation of reproduction in this species (Hanssen 1947). Following fertilization, which occurs within the first 72 h following mating-induced ovulation, the embryo develops to a blastocyst of 300-400 cells over the next four days. From fertilization through implantation, the mustelid embryo remains encapsulated in the acellular glycoprotein zona pellucida of the oocyte (Enders, Schlafke *et al.* 1986). As in other species, two components of the blastocyst are recognizable, the inner cell mass that will become the embryo proper and the trophoblast, that will become the fetal component of the placenta, and will contribute to the extraembryonic membranes. Developmental arrest ensues, and early investigations indicated that embryo growth, fluid uptake and cell replication were absent during diapause (Baevsky 1963; Daniel 1967).
The length of diapause can vary substantially between animals, with periods as brief as a few days to more than 40 days under certain experimental conditions (Murphy and James 1974). Embryo survival rates, and thus numbers of offspring, are believed to be inversely related to the length of diapause. This provides a compelling rationale for the investigation

of the mechanisms of developmental arrest, embryo reactivation and early implantation. Herein we discuss the events and potential regulation of the escape of the mink embryo from diapause and its consequent implantation into the uterus.

Materials and Methods

Animals were bred to two fertile males according to usual commercial farming procedures. To investigate the termination of diapause, embryos were collected from mated mink at 7-9 days after the final mating, or at intervals through the 10 days preceding implantation from animals treated with 1 mg/kg/day ovine prolactin (Sigma, Oakville ON) to terminate preimplantation delay. Embryos were collected by flushing of the uterine horns as previously described (Moreau, Arslan *et al.* 1995). Some embryos from each collection date were incubated overnight in the presence of 100 µM bromodeoxyuridine 5'-triphosphate (BrdU, Sigma Chemicals) to determine DNA synthesis through embryo re-activation. Following removal of the capsule, embryos were incubated with anti BrdU antibodies, and nuclei undergoing active DNA synthesis were visualized by means of a fluorescein isothionate labeled second antibody. To estimate protein synthesis, newly flushed embryos were incubated in TC-199 medium in the presence of 10 mCi/ ml ^{35}S-L-methionine (New England Nuclear, Guelph ON) at 37 C for 2 h. The incorporation of ^{35}S-methionine incorporation was determined as described by Bell et al. (1997).

Groups of five mink embryos in diapause or 9 days after activation were incubated in 500 µl INRA Menezo B2 medium (Pharmascience, Paris, France) supplemented with 5% FBS (Gibco) for 48h, with or without mink uterine epithelial cells (Moreau, Arslan *et al.* 1995). An aliquot of 100 µl of the embryo conditioned medium was used to evaluate the concentrations of PGE_2 by radioimmunoassay, according to the method described in (Xiao, Liu *et al.* 1998) using PGE_2 antiserum from Assay Design (Ann Arbor, MI). It had a percentage of cross-reactivity with PGE, $PGF_{1\alpha}$, $PGF_{2\alpha}$ and 6-keto-$PGF_{1\alpha}$ of 70%, 1,4%, 0,7% and 0.6% respectively. The assay sensitivity was 4 pg/ 100 µl. The intra-assay variation calculated between duplicates, ranged from 0.04 and 7.17%.

Embryos taken at various times after initiation of embryo reactivation were dissected and placed in separate cultures of the trophoblast and the inner cell mass (ICM) on a mouse fetal fibroblast feeder monolayer. The entire ICM was plated in mouse embryonic stem cell medium (Betts, Bordignon *et al.* 2001), while trophoblast was cultured in trophoblast stem (TS) cell medium (Tanaka, Kunath *et al.* 1998). Confirmation of trophoblast lineage was achieved by RT-PCR amplification of the marker genes Cdx2, Eomes, FGFR2, and Hand1 over time in culture in trophoblast cells derived from embryos taken at day 5 after activation. To estimate trophoblast proliferation rate, we harvested vesicular outgrowths of the trophoblast monolayer and replated them in fibroblast-conditioned TS medium in the presence and absence of 25 µg/ml FGF4. Following incubation, cells were stained with 4’-6-diamino-2-phenylindol (DAPI, Sigma Chemicals) and counted under fluorescence microscopy.

For RT-PCR amplifications, all the collected tissues were frozen in liquid nitrogen, and subsequently disrupted in RLT buffer (Qiagen, Mississauga, ON, Canada) with 0,12M -Mercaptoethanol (Sigma). RNA was purified using an RNeasy Protect Mini Kit (Qiagen) as recommended by the manufacturer, or with minor modifications to the protocol for the single blastocysts (Desmarais, Bordignon *et al.* 2004). For the uterine tissue 1.5 µg/ sample of total RNA was used for reverse transcription (RT) with Omniscript RT kit (Qiagen), according to the instructions of the manufacturer. For the blastocysts and the ICM and trophoblast cells, isolated RNA was reverse transcribed into cDNA with Superscript Rnase H- (Invitrogen, Carlsbad, CA) following the manufacturer instructions. Primers for FGF4, Cdx2, Eomes, FGFR2, PGE synthase and PGE receptors EP-2 and EP-4 were designed based on homologous sequences of human and mouse. Mink-specific primers for glyceraldehydes 3-phosphate dehydrogenase (GAPDH) were used as a control. 1 U of Taq polymerase (Amersham Biosciences Corp., Baie d’Urfe, QC, Canada) per microliter of reaction was used to amplify the cDNA from each sample. PCR products were separated in 1.2% agarose gel and stained with ethidium bromide for visualization. At least three independent samples were sequenced for verification of transcript identity.

A mink ovarian tumor cell line was used to test the effects of PGE on VEGF promoter transactivation. A 2 Kb construct of the proximal mink promoter region

was used in a luciferase reporter assay. Briefly, the proximal promoter region was inserted in a pGL2 vector and mink cells were transfected using the Effectene reagent (Qiagen). Transfected cells were then treated with 10µM PGE (Sigma) for 24 h. Renilla luciferase control vector pRL.SV40 was used to normalize results for transfection efficiency.

Data were analysed by means of least square analysis of variance in the General Linear Model procedures of SAS. Following confirmation of a significant F value, comparisons among means were made by the Tukey HSD test. Regression analysis was performed for in vitro proliferation experiments. Significance was established at $p<0.05$.

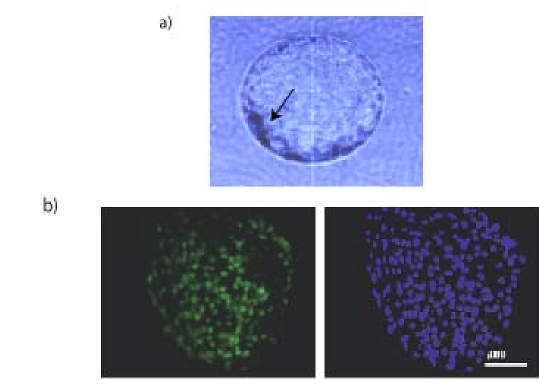
Results

Embryos in diapause displayed the carnivore phenotype including the presence of the oocyte derived capsule, the trophoblast and an inner cell mass (Fig. 1a). They did not take up BrdU, indicating an absence of significant DNA synthesis. At 72 h after the initiation of activation, most cells in the embryo were positive for BrdU, and thus, in the S-phase of the cell cycle (Fig. 1b). Although there appeared to be embryo expansion within 24 h after activation, the first statistically definable increase was present at 72 h, and there was a consequent increase in embryo diameters through day 11 (Fig. 1c).

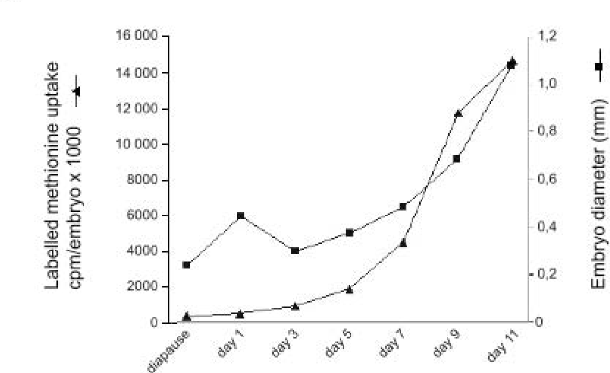
The embryos found in the uteri at day 13 after initiation of reactivation in approximately half of the treated animals were attached and undergoing implantation (data not shown). The onset of protein synthesis occurred somewhat approximately 24 h following initiation of expansion, while the first discernable increase in the 35S-methionine uptake was evident at 96 h after the initiation of reactivation (Fig. 1c). Embryos collected at subsequent times displayed large scale increases in protein synthesis from days 7-11 (Fig. 1c).

Neither ICM nor trophoblast cells taken from embryos in diapause were capable of proliferation in vitro. Trophoblast cells proliferated readily at day 5 after embryo activation while ICM colonies grew more quickly at day 9 (data not shown). FGF4 is believed to be expressed by the ICM and to affect the proliferation of the trophoblast. RT-PCR analysis revealed that FGF4 transcripts were present in the embryo from day 3 after initiation of activation (Fig 2a). FGF receptor mRNA first appeared on day 5

Figure 1(a). The mink embryo in diapause showing the capsule and the trophoblast. The darkened cellular mass at 8 o'clock is the inner cell mass (arrow).
(b) The mink embryo at day 3 after reactivation showing BrDU uptake (left panel) and DAPI staining to identify nuclei (right panel).
(c) Concurrent increases in embryo volume as measured by embryo diameter and embryo protein synthesis as indicated by ^{35}S-methionine uptake following embryo activation.

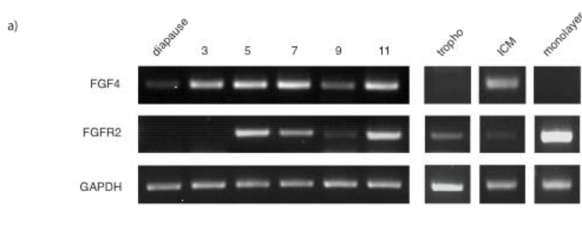


c)



after reactivation (Fig 2a) and both transcripts persisted through implantation. Examination of trophoblast and ICM cultures indicated that the latter was the source of FGF4, and that the trophoblast expressed the receptor (Fig 2a). Trophoblast proliferation in vitro was dependent on the presence of FGF4 (Fig. 2b).

Figure 2 (a). RT-PCR evaluation of embryo expression of fibroblast growth factor 4 (FGF4) and its cognate receptor in whole embryos during reactivation (diapause through day 11) and in trophoblast, ICM and fibroblast feeder layer cultures.
(b) Proliferation of trophoblast cells in vitro in the presence or absence of FGF4.



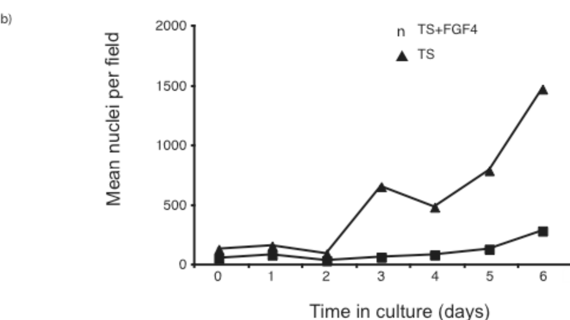
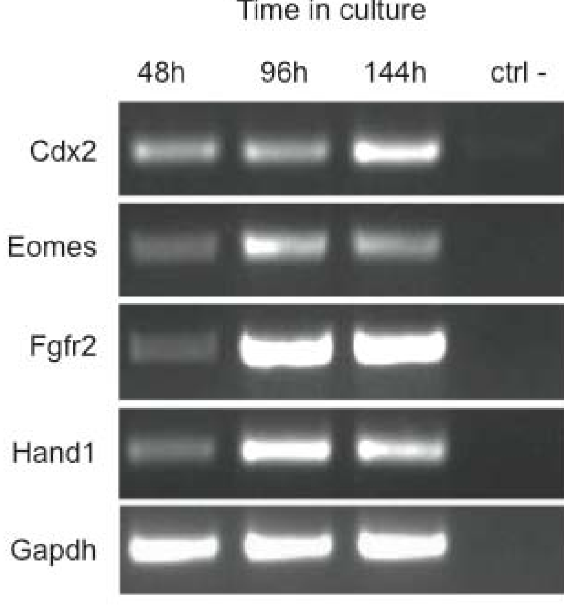


Figure 3. Semi-quantitative RT-PCR analysis demonstrating the evolution of the occurrence of markers for trophoblast during embryo reactivation. Trophoblast cells were separated from the ICM and cultured for 48, 96 and 144 h prior to isolation and reverse transcription of RNA.



A series of expression markers were examined to establish whether cell lines derived from mink embryos were, in fact, derived from the trophoblast (Fig. 3).

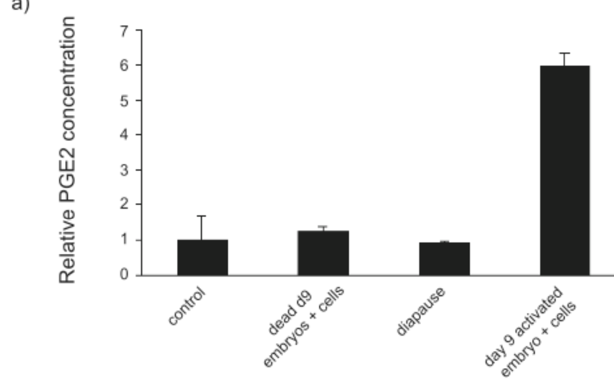
The results demonstrate that all markers, with the exception of Pal31 are present in low abundance in recently derived cell cultures, there is significant upregulation of the expression of each over time. The most prominent increase was in FGFR2, the putative effector of FGF4 effects on trophoblast proliferation.

We then undertook to determine if embryos in diapause had the capability to synthesize and secrete molecules to signal the uterus. Figure 4a depicts the accumulation of PGE_2 in medium of cells (control) and embryos cocultured with cells over 48 h of incubation. It reveals that embryos collected in diapause had no apparent capability to synthesize and secrete PGE_2. Embryos harvested on day 9 after reactivation had six-fold greater PGE_2 secretion relative to diapause embryos, cells alone, or dead embryos. To examine whether cells were required for PGE_2 synthesis, embryos were incubated alone (Fig4b).

The results indicate that the source of the prostaglandin synthesis is the live embryo, as concentration of the hormone is several fold greater in medium containing embryos.

To further explore the potential for embryo signaling to the uterus of the mink, we identified mRNA for PGE synthase, and for two of the PGE_2 receptors of the EP subtype (EP-2 and 4) in the postimplantation mink uterus (Fig 5a). We then tested the potential for PGE_2 to affect the transcription of VEGF, an important downstream gene in placental development. The results indicated that PGE_2 treatment induced a precipitous increase in the VEGF promoter activity (Fig. 5b).

Figure 4 (a). Accumulation of prostaglandin E2 (PGE_2) in media derived from incubation for 144 h of mink embryos in diapause or following activation. Embryos showing cell death have been included for comparison. Embryos were cultured over a layer of mink uterin stromal cells.
(b) Incubation of embryos in the absence of the feeder layer of uterine cells for 144 h resulted in several-fold increases in $PGE_2$2 accumulation.



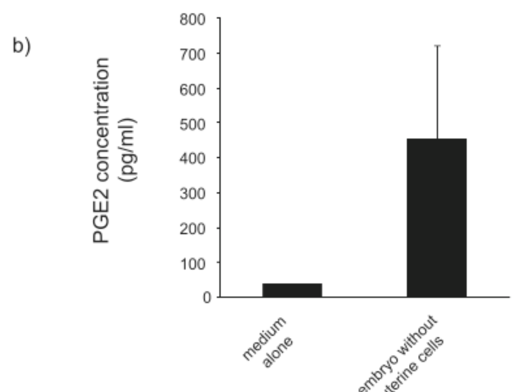
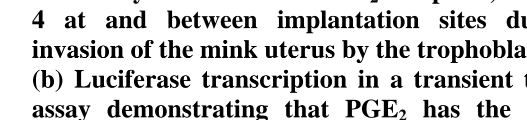
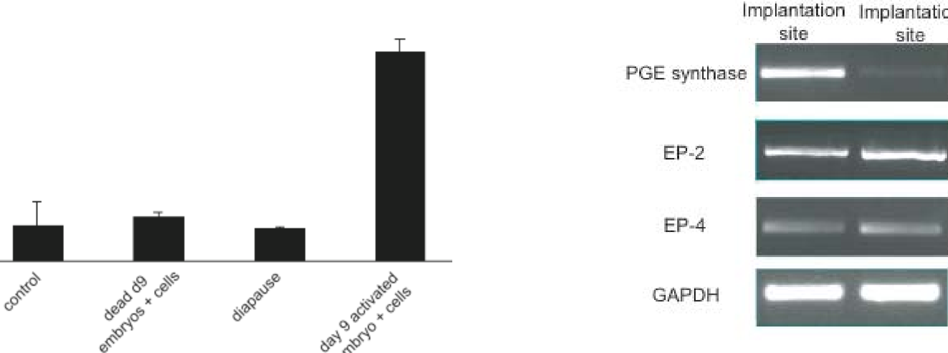
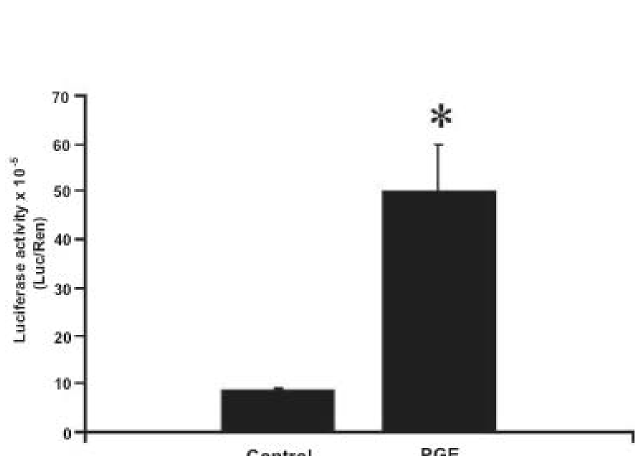


Figure 5 (a). RT-PCR analysis of transcript abundance of PGE-synthase and the PGE_2 receptors, EP-2 and EP-4 at and between implantation sites during early invasion of the mink uterus by the trophoblast.
(b) Luciferase transcription in a transient transfection assay demonstrating that PGE_2 has the capacity to stimulate promoter activity of the mink VEGF gene in mink cells.







Discussion

The results of the present study support the long held view that the cells that comprise the mink embryo in diapause have exited from the cell cycle. It is only after reactivation that DNA synthesis occurs in the trophoblast and the ICM. It is of interest to note that cells derived from either embryonic compartment during diapause are likewise incapable of proliferation in vitro. It is known that the maternal environment influences that persistence of diapause (Chang 1968), indicating that the uterus either prevents reactivation or the uterus during diapause lacks elements that allow for the continuation of embryonic development. It is known that growth of embryos collected in the diapause state can occur in vitro, in the absence of maternal signals (Moreau, Arslan *et al.* 1995), providing support for the view that maternal inhibition maintains diapause. This notwithstanding, acceleration of development can be induced in

embryos recovered after reinitiation of development (0.4 mm or greater) by prolactin (Polejaeva, Reed *et al.* 1997), which argues for positive control by maternal factors.

In fact, the complex pattern of events that bring about the reactivation, trophoblast attachment to the uterus and consequent invasion of the trophoblast in the implantation process remains largely undetermined. We have endeavored to assemble some of the pieces of the puzzle based on the hypothesis that signaling between cells in the embryo and between the embryo and the uterus induces embryonic maturation and postimplantation events in the uterus. In the first instance, we have shown that activation induces early increases in both embryo volume and embryo protein synthesis. Activation is also characterized by expression of FGF4, and the acquisition of its cognate receptor by the embryo. In vitro studies with trophoblast cells indicate that, as in the mouse (Tanaka, Kunath *et al.* 1998), FGF4 is an important stimulator of proliferation. In the mouse, it has been shown that FGF4 is expressed by the ICM (Yuan, Corbi *et al.* 1995). Our results support this view, in that FGF4 expression was restricted to ICM cultures while the FGF4 receptor was found in trophoblast cells. We suggest that the ICM of the mink embryo expresses FGF4 early in activation and signals the trophoblast causing the preimplantation proliferation that we have observed.

The significance of the cyclo-oxygenase (COX) enzymes that synthesize prostaglandins to embryo implantation has been demonstrated in a number of species (Das, Wang *et al.* 1999). We have shown that the mink embryo and endometrium express COX-2, the regulated version of the rate limiting enzyme for prostanoid synthesis at the time of embryo attachment (Song, Sirois *et al.* 1998). The present results indicate that the activated embryo synthesizes PGE_2, independent of uterine influence. RT-PCR amplification of transcripts from uterus at the time of implantation revealed that attachment and invasion sites, but not inter-implantation sites express PGE-synthetase, the key enzyme in PGE_2 synthesis. Further, the transcripts for the PGE_2 receptors, EP1 and EP2 were abundant in the early postimplantation uterus. Together these results argue for signaling by the activated and early implanted embryo to the uterus as a component of the attachment and invasion process.

There is a growing body of evidence to indicate that eicosanoids are important regulators of angiogenesis in a number of tissues, including the uterus (Fujiwaki, Iida *et al.* 2002). Given that PGE_2 appears to be synthesized by the activated mink embryo and by the trophoblast at the site of implantation, it was deemed interesting to determine whether PGE_2 could induce in vitro transcriptional activation of the principal angiogenic element, VEGF. Our results, using the gene promoter from mink VEGF transiently transfected into mink cells demonstrate a marked PGE_2 stimulation of transcription.

In summary, we have investigated the potential signaling cascades that regulate the proliferation of the mink trophoblast, its invasion, and its capacity to induce angiogenesis. While much of the overall pattern remains unresolved, the present investigation indicates that embryo reactivation engenders synthesis of FGF4 by the embryo which has a paracrine effect on proliferation of the trophoblast. The activated embryo also secretes PGE_2, both before and after implantation, and this hormone has the capacity to induce angiogenic factors that are important to the invasion process. The embryo-uterine signaling in embryonic diapause, embryo reactivation and implantation is a fertile ground for further investigation.

References

Baevsky UB (1963) The effect of embryonic diapause on the nuclei and mitotic activity of mink and rat blastocysts. In 'Delayed Implantation'. (Ed. AC Enders) pp. 141-153. (University of Chicago Press: Chicago)

Bell JC, Smith LC, Rumpf R, Goff AK (1997) Effect of enucleation on protein synthesis during maturation of bovine oocytes in vitro. *Reprod Fertil Dev* 9, 603-8.

Betts D, Bordignon V, Hill J, Winger Q, Westhusin M, Smith L, King W (2001) Reprogramming of telomerase activity and rebuilding of telomere length in cloned cattle. *Proc Natl Acad Sci U S A* 98, 1077-82.

Chang MC (1968) Reciprocal insemination and egg transfer between ferrets and mink. *J. Exp. Zool.* 168, 49-60.

Daniel JC, Jr. (1967) Studies on the growth of the mink blastocyst. *J Embryol Exp Morphol* 17, 293-302.

Das SK, Wang J, Dey SK, Mead RA (1999) Spatiotemporal expression of cyclooxygenase 1 and cyclooxygenase 2 during delayed implantation and the periimplantation period in the Western spotted skunk. *Biol Reprod* 60, 893-9.

Desmarais JA, Bordignon V, Lopes FL, Smith LC, Murphy BD (2004) The escape of the mink embryo from obligate diapause. *Biol Reprod* 70, 662-70.

Enders AC, Schlafke S, Hubbard NE, Mead RA (1986) Morphological changes in the blastocyst of the western spotted skunk during activation from delayed implantation. *Biol Reprod* 34, 423-37.

Fujiwaki R, Iida K, Kanasaki H, Ozaki T, Hata K, Miyazaki K (2002) Cyclooxygenase-2 expression in endometrial cancer: correlation with microvessel count and expression of vascular endothelial growth factor and thymidine phosphorylase. *Hum Pathol* 33, 213-9.

Hanssen A (1947) The physiology of reproduction in the mink (*Mustela vison* Schreb.) with special reference to delayed implantation. *Acta Zool.* 28, 1-136.

Moreau GM, Arslan A, Douglas DA, Song J, Smith LC, Murphy BD (1995) Development of immortalized endometrial epithelial and stromal cell lines from the mink (Mustela vison) uterus and their effects on the survival in vitro of mink blastocysts in obligate diapause. *Biol Reprod* 53, 511-8.

Murphy BD, James DA (1974) The effects of light and sympathetic innervation to the head on nidation in mink. *J Exp Zool* 187, 267-76.

Polejaeva IA, Reed WA, Bunch TD, Ellis LC, White KL (1997) Prolactin-induced termination of obligate diapause of mink (Mustela vison) blastocysts in vitro and subsequent establishment of embryonic stem-like cells. *J Reprod Fertil* 109, 229-36.

Song JH, Sirois J, Houde A, Murphy BD (1998) Cloning, developmental expression, and immunohistochemistry of cyclooxygenase 2 in the endometrium during embryo implantation and gestation in the mink (Mustela vison). *Endocrinology* 139, 3629-36.

Tanaka S, Kunath T, Hadjantonakis AK, Nagy A, Rossant J (1998) Promotion of trophoblast stem cell proliferation by FGF4. *Science* 282, 2072-5.

Thom MD, Johnson DD, MacDonald DW (2004) The evolution and maintenance of delayed implantation in the mustelidae (mammalia: carnivora). *Evolution Int J Org Evolution* 58, 175-83.

Xiao CW, Liu JM, Sirois J, Goff AK (1998) Regulation of cyclooxygenase-2 and prostaglandin F synthase gene expression by steroid hormones and interferon-tau in bovine endometrial cells. *Endocrinology* 139, 2293-9.

Yuan H, Corbi N, Basilico C, Dailey L (1995) Developmental-specific activity of the FGF-4 enhancer requires the synergistic action of Sox2 and Oct-3. *Genes Dev* 9, 2635-45.

IV – 7 RP

The effects of air pollutants on the cortisol and progesterone secretion in polar fox (*Alopex lagopus*)*

Bożena Nowakowicz-Dębek, Leon Saba, Hanna Bis-Wencel
Section of Reproduction Biology, Department of Animal and Environmental Hygiene,Faculty of Biology and Animal Breeding, University of Agriculture in Lublin, 13 Akademicka Str., 20-950 Lublin, Poland, e-mail: nowak@ursus.ar.lublin.pl

* This work was conducted as part of the research project no. 3 PO6Z 054 24 financed by the State Committee for Scientific Research

Abstract

The aim of the present work was to show the effect of air pollution on a level of cortisol and progesterone at blue foxes on 25-35 days following the mating. The animals from a farm situated in the south eastern Poland were maintained at the pavilion system and constituted the control. The experimental group was made up by the females kept in the chamber with limited air movement, thus exposed to air contaminants. The air monitoring confirmed the occurrence of higher concentrations of gaseous substances in the chamber. In the females exposed to the air pollutants there were recorded higher levels of the hormones released. The dams exposed to the pollutants showed higher levels of the hormones secreted. The mean values of progesterone in the females of the experimental group were 72,32 ng/ml, whereas in the control – 42,62ng/ml. The mean values of cortisol in the experimental group recorded were 210,88nmol/l and proved to be substantially higher than in the control. This fact proves the activation of the defensive mechanisms as well as the impact of exogenous agents. The statistical analysis involved the test of double cross classification.

Introduction

Polar foxes (*Alopex lagopus*), commonly called the blue, have been "domesticated" for nearly one hundred generations. The appropriate environmental conditions were created for them so they could live and reproduce. Through the several-stage selection process the individuals were obtained with highly functional qualities and decreased susceptibility to stress. These assumptions have been continued by means of the breeding methods improvement, providing a high level of animal welfare as well as a proper contact between man and animal. Under the undesirable environmental conditions, apart from the stress resulting from welfare depression, there is recorded inactivation of, among others, the hypothalamus-adrenal system. The presence of generally perceived stressors may lead to some changes in physiology, reproduction and behaviour as shown in the investigations carried out at the laboratory animals [Braastad et al, 1998; Nowakowicz-Dębek et al, 2003 and 2004; Smith, 1998].

The aim of the following work is to determine the effects of air pollutants on the level of cortisol and progesterone at foxes.

Material and methods

The investigations were performed at a polar fox (*Alopex lagopus*) farm situated in the south-eastern part of Poland. The animals were caged in open air according to the pavilion system made the control (group A). To show the influence of released air contaminants on foxes, a female group was placed in a chamber with limited air movement (ranging from 0,1 to 0,2 m/s), yet at permanent outside air inflow and outflow (group B). Throughout the experimental period the animals were provided with veterinarian and zootechnical service; air quality was monitored by gas chromatography and colorimetric techniques [Nowakowicz-Dębek et al, 2003; Rodel and Wolm, 1992].

Blood for the cortisol and progesterone determination was collected from the female foxes on 20-35d following the mating. The material for cortisol analysis was taken for not longer than 2-3 minutes. At both cases blood was collected from the foot vein (*vena saphena parva*), simultaneously in every group. The level of the mentioned indices was fixed by the immunoenzymatic method with the kits from BioMerieux.

The statistical analysis was performed with the double cross classification test.

Results

The feeding conditions and animal maintenance are vital for their health state, efficiency and

reproduction parameters. Air pollution as one of the microclimate factors induces the disturbance of animal homeostasis and as a consequence, a decline of animal welfare. One of the systems affected by these changes is, beside the immune system, the neurohormonal system. Therefore, apart from hormone secretion measurements, air monitoring is carried out. Air chromatographic and colorimetric analysis showed higher levels of gaseous pollutants in the chamber compared to the farm. Fairly high levels were recorded for ammonia and sulphur compounds (especially mercaptans, sulphides).

Tab.1. Mean levels of sulphur compounds and ammonia over the analyzed period

Name of compound group	Farm ($\bar{x}$ ±SD)	Chamber
Inorganic sulphur compounds (μg/m^3)	0,75± 0,30	1,86± 1,65
Mercaptans (μg/m^3)	2,06± 1,00	19,31± 26,80
Sulphides (μg/m^3)	0,73± 0,42	1,16 ± 1,11
Ammonia (mg/m^3)	0,37± 0,17	1,37± 1,66

$\bar{x}$ *- the mean*
SD – standard deviation

A part of the analysed and published findings was also presented in Fig.3, where high concentrations of phenol, ethylbenzene, naphthalene, methane and other gases were shown in the chamber [Nowakowicz-Dębek et al, 2003 and 2004]. The chamber microclimate differed markedly from the conditions recorded at the farm.
In the examined foxes the progesterone level was differentiated on 20-35d after mating. In the females maintained at the chamber, its mean values were substantially higher (72.32 ng/ml) as against the control (farm-42.63 ng/ml). The circulating levels of progesterone observed at the successive collections varied between the examined groups.
The I collection exhibited a marked progesterone increase at the foxes from the experimental group (B) compared to the control (A). Progesterone release at II collection, however, decreased and the difference was greater than in the experimental group.

Fig.1.Progesterone levels at dams at the successive collections at the farm and chamber (ng/ml)

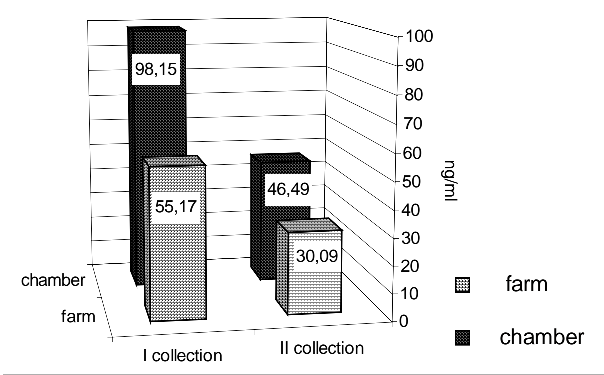
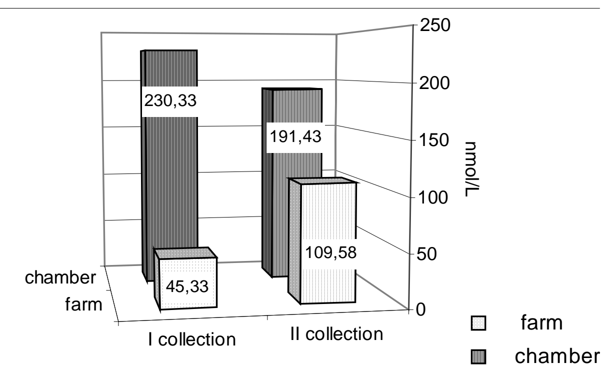
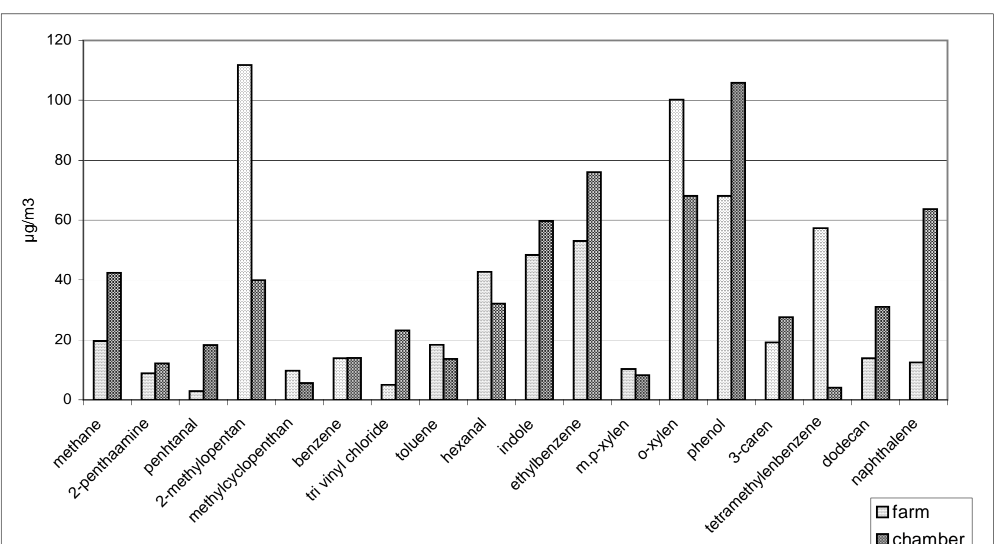


Fig.2.Cortisol levels at dams at the successive collections at the farm and chamber (nmol/L)



The cortisol values from both fox treatment groups are presented in Fig. 2. The highest values were detected for the foxes kept in the chamber (mean – 210.88 nmol/L), i.e. those exposed to gaseous pollutants (Fig. 3).
At successive collections, different fluctuations of cortisol were reported. In group A, its mean values had an upwards tendency at the successive collections, whereas in B a decline was noted. Comparing the mean values of cortisol of the foxes from group A and B some substantial differences were recorded subject to a collection. In I collection it reached 230 nmol/L, whereas at II – 81,85 nmol/L; the differences were statistically significant. In the face of the fact that the air examinations exhibited a higher level of gaseous pollutant release in the chamber in relation to the farm, an attempt was made to study an organism response to the harmful compound activity. Basing on the above mentioned data it may be assumed that the differences in the secretion of particular hormone might have been induced by an experimental factor, that is air pollution.

Fig.3.Mean values of other gaseous compounds identified at the farm and chamber in μm³ [acc. to Nowakowicz-Dębek et al., 2004]



Discussion

The optimum breeding and rearing conditions is an essential requirement to show balance in the environment and animal welfare.

The investigations performed revealed the environment-animal relations. By means of depression and change of the basic microclimate parameters a tendency was to exhibit occurrence of disturbances in animal organism, particularly in the first trimester of pregnancy when dam sensitivity increases and embryos are implanted. A hormone essential for pregnancy maintenance proves to be progesterone and blocking its activity results in abortion. It is also indispensable to provide the appropriate development of the uterus mucosa that constitutes the base for implantation and new organism growth. Like estrogens, it affects the cells and tissues of whole organism, stimulating their metabolism. The pregnancy time is often termed a period of profound hormonal changes that manifest themselves with fluctuations of levels and hormone metabolism. An increase of sex hormones lead to, among others stimulation of respiration and diastole of bronchi, because progesterone is a stimulator of this center in the central nervous system. In the case of the respiratory system impairment progesterone can be used as an agent stimulating the ventilation, yet the researches have not been confirmed [Braastad et al, 1998; MnLean et al, 1995; Schatz, 1999; Smith, 1998].

The exhibited great differences in the progesterone in circulation indicate the influence of the environmental factors and inactivation of defensive mechanisms preventive of various disturbances. However, the present state of research does not allow to explain or to indicate the initiators of the disorders. The identified compounds are, among others, xenobiotics that may lead to the changes in organism functioning. Here the toxic operation of ammonia should be mentioned as it is present in the chamber air. Prolonged exposure to ammonia may lead, among others to some damage and paralysis of the respiratory center. Whereas, exposition to contaminants, like sulphur compounds recorded in the chamber air should be considered as the respiratory tracks irritation or even disturbances of the peripheral and central nervous system. The toxic impact of many pollutants like that have been documented, whereas no works available discussing their influence on a hormone release mechanism, at pregnancy in particular. It should be pointed out that in the polluted farm air there appears full mixture of harmful gases. It is not expected to analyse them separately because as it is well known their operation in the gas mixture can get enhanced or attenuated.

Another important mediator in organism proves to be cortisol. It is often considered a stress hormone occurring at endosystemic disturbances to mobilize organism. It shows, among others anti-inflammatory

activity, increased lymphocyte count in blood, inhibits immune response as well as reaction of allergen and antibody that inhibits allergic reaction. Cortisol, being a hormone of adrenal cortex, also affects the reproduction system. Generated by the fetal adrenal gland, it stimulates lungs development and maturity. Beside, it is one of factors of the parturition-inducing mechanism [Kazimierczak, 2001; McLean et al 1995; Smith, 1998].

In numerous works concerning behavior and the parturition mechanism of animals the authors refer to the secretion of cortisol. Osadchuk et al. [2003] reports that improper conditions of blue fox maintenance during pregnancy or stress brought about by inappropriate service may be manifested with an increase of cortisol secretion in dam`s adrenal gland or fetus plasma cortisol. Dam`s stress results in disturbances of sex steroid formation at fetuses. The changes were reported to appear especially at the morphometrical measurements of female fetuses [Osadchuk et al, 2003]. The prenatal stress effects of blue fox cubs were also described by Braastad et al. [1998]. The authors indicated the postnatal consequences of its activity on dam`s organism over the last trimester of pregnancy.

The glucocorticoid levels change under various stress forming situations. Rekila et al. [1999] claim that elevated response is differentiated and depends on a species as well as individual psychic constitution. The authors imply that there is a broad individual variation, which is significant at animal selection for farm population.

Hormones of a developing organism regulate metabolism, control the metabolic processes and homeostasis, i.e. stability of the internal conditions crucial for animal life. The disturbance of this equilibrium caused by the undesirable conditions of microclimate is likely to have negative effects on animals as it has been demonstrated in the present work.

References:

Braastad B.O., Osadchuk L.V., Lund G., Bakken M.: Effects of prenatal handling stress on adrenal weight and function and behaviour in novel situations in blue fox cubs (Alopex lagopus). Applied Animal Behaviour Science 57, 157-169, 1998.

Kazimierczak A.: Postępowanie u kobiet w ciąży chorujących na astmę oskrzelową. Postępy Nauk Medycznych, 1, 1-5, 2001.

McLean M., Bisits A., Davies J., Woods R., Lowry P., Smith R.: A placental clock controlling the length of human pregnancy. Nature Medicine, 1(5), 460-463, 1995.

Nowakowicz-Dębek B., Bis-Wencel H., Molenda-Pyzik M., Likos B., Wnuk W.: Emisja niektórych związków organicznych przez fermę lisów z uwzględnieniem uwarunkowań środowiskowych. Zeszyty Naukowe Przeglądu Hodowlanego, 68 (6), 169-177, 2003.

Nowakowicz-Dębek B., Saba L., Bis-Wencel H., Wnuk W.: Uwalnianie lotnych substancji gazowych w zależności od warunków utrzymania lisów polarnych (*Alopex lagopus*). Annls. Univ. Mariae Curie-Skłodowska, 2004, sec. EE (w oprac.red.).

Osadchuk L.V., Braastad B.O.,Hovland A.L.,Bakken M.: Handling during pregnancy in the blue fox (alopex lagopus): the influence on the fetal gonadal function. General & Comparative Endocrinology. 2003, 132 (2), 190-197.

Rekilä T., Harri M., Jalkanen L., Mononen J.: Relationship Between Hyponeophagia and Adrenal Cortex Function in Farmed Foxes. Physiology&Behavior, 65, 4/5, 779-783, 1999.

Rodel W., Wolm G.: Chromatografia gazowa. Wyd. PWN, Warszawa 1992

Schatz M.: Interrelationships between asthma and pregnancy: Aliterature review Journal Allergy Clin. Immunolog., 103, 2p2, 330-336, 1999.

Smith R.: Corticotropin-releasing hormone directly and preferentially stimulates dehydroepiandrosterone sulfate secretion by human fetal adrenal cortical cells. Journal of Clinical Endocrinology and Metabolism, vol. 83, 8, 2916-2920 VIII/1998.

IV – 8 P

The influence of antioxidant emicidin on minks' physiological condition and reproduction

Irina S. Sugrobova, Tatiana M. Demina*, Olga V. Rastimechina*,*
Elena A. Tinaeva, Vladimir I. Melnichenko***
** V.Afanasiev Research Institute of Fur Bearing Animals and Rabbits*
Ramensky District, Moscow Region, Rodniki 140143, Russia;
*** Triniti farma, Moscow region, Russia*
E-mail: NIIPZK@orc.ru

Abstract

Antioxidant emicidin has an expressed quality to connect free radicals, it stabilizes cells' membranes and helps to increase the indices of animals' productivity. Emicidin was injected (enterally and orally) in doses 7, 25 and 50 mg an animal a day.
It was found that emicidin assists to increase the safety of cubs at the rate of 7-16 % and has growth assisting effect (males and females both). It also helps to increase, has growth the quality of skins by 5-17 % in males. Emicidin also normalizes the level of general protein of blood serum in whelped female minks, increases their lactation and absolutely excludes females' lactational exhaustion. The strongest effect of using antioxidant emicidin was checked when injecting it orally to mature females in dose 25 mg an animal a day during the periods of whelping and lactation.

Introduction

In modern animal industries biologically active substances with antioxidantal properties are beginning to find wide using. One of the substances with the expressed ability to connect free radicals, to inhibit processes of peroxidal oxidation of biomembranes' lipids and to reduce intensity of oxidizing processes in organism is emicidin.
Emicidin - 2 ethil, 6- methyl, 3-oxipiridin sukcinate represents a crystal white powder with a cream shade, easily soluble in water, with pH from 4,3 up to 4,9.
Due to the mechanism of action, emicidin has a wide spectrum of pharmacological effects and influences basic key parts of pathogenesis of various diseases associated with processes of free radical oxidation.
There was found growth stimulating and antistressful action of emicidin on pets' organism.
Results of our preliminary researches on application of emicidin in minks breeding indicate its positive influence on a number of productive parameters of animals when injected enterally.
Therefore there is a doubtless scientific and practical interest in the further studying of influence of antioxidant emicidin on viability of females minks weakened due to of lactation, especially having many cubs and also on the growth of suckling and young minks, taken from mothers.
The present research was carried out with the purpose of receiving objective estimation of efficiency of use of antioxidant emicidin in minks breeding.

Materials and Methods

Researches were conducted on sapphire and brown minks of wild type.
In experiments on the base of «Rodniki» of the Moscow region (Russia) there were used 280 lactating females and 1768 cubs received from them.
There was estimated influence of antioxidant emicidin on physiological
condition and productive parameters of minks in the various biological periods: lactation (April - June), active growth of young animals (June - August), at various ways of injection (peroral, parenterally) in dozes 7, 25 and 50 mg on a head per day under the developed circuit.
Experiments were executed according to the accepted requirements on formation of experimental groups, maintenance and feeding of animals (Balakirev & Yudin, 1994).
Biochemical and patomorphological researches were conducted with the use of standard methods (Berestov, 1976).
The following parameters were used as criteria of an estimation of animals' physiological condition: viability of females and safety of cubs, concentration of the general protein of blood serum, the size of adrenal glands of cubs at slaughter.

Efficiency of animals was estimated by parameters of absolute and relative gain of body weight of suckling (males and females both) and young animals (males), taken from mothers.

Results and Discussion

In the present work results of 5 experiments are used.

Experimental data have allowed to define positive influence of antioxidant emicidin on physiological condition and productivity of minks, receiving various dozes of antioxidant with feed composition or parenterally, both during the reproductive period and during active growth of young minks (Table 1).

Results of researches have shown, that injection of emicidin caused the increase of milk yield and, as a result, the increase of safety of cubs of minks' females with many cubs on 10-12 % . Animals were injected by antioxidant first ten days of lactation.

The relative gain of body weight of suckling cubs to 20-day's age was higher on 4-5 % and cubs were more viable.

The parameters of safety received by parenterally and peroral ways of injection of emicidin were similar in efficiency, at the same time, the second method of injection appeared to be less toilful and, therefore more acceptable for technology of fur farming.

Most of experimental researches were carried out with the use of this way of injection of antioxidant.

Positive influence of emicidin on the viability of lactating females is confirmed. It has ability to prevent the exhaustion of females in lactational period whereas 12% of intact females perish with the diagnosis «lactational exhaustion».

Safety of suckling young animals receiving emicidin by the time of taking them from females has received 84,7-94,8 % against 69,0-88,2 % - in the case of intact females. The best effect was in the group with doze 25 mg / head.

Table 1 The results of using emicidin (united data of 5 experiments)

Age group	Number of animals	Dose mg	Indexes	Methods of injection			
				parenterally	control	peroral	control
Suckling cubs	458	25	Safety by taking from mother, %	88.3	76.6-78.4	85.4	69.0
		25	Average increase of body weight by 20 day	165	160-161	-	-
	1483	7	Safety by taking from mother, %	-	-	87.1	88.2
		25		-	-	85.4-94.8	69.0-88.2
		50		-	-	84.7	69.0
		7	Including from litters with 7-11 cubs	-	-	87.0	84.2
		25		-	-	85.4-94.4	69.0-84.2
		50		-	-	84.7	69.0
Mature females	217	7	Safety after taking cubs, %	-	-	100	88.2
		25		-	-	100	88.2
		7	Including females with many cubs	-	-	100	76.3
		25		-	-	100	76.3
Cubs taken from mothers	216	7	Average increase of body weight, %	-	-	98.5	100.5
		25		-	-	106.2	100.5
		50		-	-	95.9	91.9
	216	7	Skins without defects, %	-	-	14.8	0
		25		-	-	14.8	0
		50		-	-	22.9	0
	216	7	Quality index	-	-	94.8	88.8
		25		-	-	89.4	88.8
		50		-	-	99.3	82.2

Influence of antioxidant was best revealed on females having 7-11 cubs. In this group no female died after the cubs were taken from them, while the safety of intact females was 76,7 % only.

Viability of cubs of experimental females with many cubs was much higher, than in the control - 84,7-94,4 % against 69,0-84,2 %. At the same time best result was taken from females, receiving emicidin in a doze 25 mg / head.

There was marked more intensive growth of cubs taken from mothers, receiving emicidin in dozes 25 and 50 mg / head in comparison with intact animals.

By the end of August (the end of intensive growth) distinctions in body weight of animals in experimental groups (on the average) and the control reach authentic value: 1766 ± 21 against 1622 ± 22 g ($P < 0,001$), gradually smoothing out to slaughter (November). The body weight was 2248 ± 47 g against 2186 ± 51 g, length of a body – 48,8 ± 0,34 sm against 48,6 ±031, accordingly.

Females, receiving emicidin, had more intensive increase of concentration of the general protein of blood serum during the first 20 days after whelping (after large blood loss). The relative gain of level of the general protein of blood serum in them in this period has made 36,8 % against 18,2 % in intact animals.

Patomorphological researches of gastroenteric system of the cubs taken from mothers receiving and not receiving emicidin during active growth, have revealed pathological changes of organs accordingly at 40 % and 60 % of the surveyed animals.

At the animals receiving emicidin, the weight of adrenal glands was less 12,5-20 %, than at intact, so it is possible to be considered as the consequence of display of antistressful action of the substance. It is corresponded with data taken by L.Osadchuk (2001) which specify the connection of the sizes of adrenal glands and level of produced corticosteroids, the production of which amplifies in reply to influence of stresses - factors.

Conclusion

The given data indicates the positive influence of antioxidant emicidin on viability of lactating females and young minks.

The greatest effect from using of antioxidant emicidin reveals during its peroral injection to the adult mink females in a doze of 25 mg on a head in a day. At the same time the most essential effect of emicidin reveals on the viability of female minks with many cubs and their cubs.

References:

Balakirev N.A. & Yudin V.K. 1994. The methodical manual on madding scientifical and practical experiments on fur animal feeding.

Berestov V.A. 1976. Biocheemical and morphological of the blood of fur bearing animals. Petrozawodsk. 292 p.

Osadchuk L. 2001. Estimation of potential prolificacy, embrional deadly and viability in silver foxes aftor long selection on domestical behavior. Zoological Journal, 80 (7), 864-870.

IV – 9 RP

The measurements of the skin electrical conductivity in the acupuncture points affecting reproduction in female polar foxes (*alopex lagopus*) during the estrus period

*Kazimierz Ściesiński, Marian Brzozowski**
Department of Animal Breeding and Production, Warsaw Agricultural University - SGGW, Ciszewskiego 8, 02-786 Warsaw, Poland
**e-mail: brzozowskim@delta.sggw.waw.pl*

Summary
The aim of the investigation was the measurement of electrical conductivity (electric potentials in µA – 1 x 10^{-6} A) in the acupuncture points in female polar foxes during diestrus and estrus.
The measurements were taken in the acupuncture points situated on the urinary bladder meridian (points B_{22}, B_{23}, B_{25}, B_{31}, B_{32}) and on the meridian of the main beck regulator (points Lg_2, Lg_3, Lg_4) which are stimulated in cases of reproduction disturbances (during parturition and postpartum period) and additionally the acupuncture points situated on the large intestine meridian (points LI_4 and LI_{11}) affecting the immune system. The mean range of the skin electrical conductivity in the chosen points during estrus amounts to 81.2 - 88.1 µA on the urinary bladder meridian (points B_{22}, B_{23}, B_{25}, B_{31}, B_{32}) and to 86.0 - 87.7 µA on the meridian of the main back regulator (points Lg_2, Lg_3, Lg_4). The values are much higher than those observed during the diestrus period ($p<0.01$).
The values of the skin electrical conductivity measured in the points situated on the large intestine meridian (LI_4 and LI_{11}) affecting the immune system amounting to 68.7 - 70.0 µA and don't differ statistically from the results during diestrus period in female polar foxes.

Introduction
The electroacupuncture diagnosis is used in human medicine and there are some attempts at using the electroacupuncture in animal production (Hyodo, 1979). The aim of the investigation was the measurement of electrical conductivity (electrical potential in µA - 1 x 10^{-6} A) in the acupuncture points affecting reproduction results in the polar foxes females during estrus and compare them with the results from diestrus period.
The measurements were taken in the acupuncture points situated on the urinary bladder meridian (points B_{22}, B_{23}, B_{25}, B_{31}, B_{32}) which are stimulated in cases of disturbances during estrus, parturition and postpartum period and on the meridian of the main beck regulator (Lg_2, Lg_3, Lg_4) which are stimulated during parturition and postpartum period. In addition the measurements of electric potential in the points situated on the large intestine meridian (LI_4 and LI_{11}), affecting the immune system stimulation were taken during estrus and diestrus periods.

Material and methods
15 females 2-4 years old, with similar condition were chosen for the experiment. The measurements of electrical potential in acupuncture points during diestrus, right before estrus (March – April) were taken. The measurements were repeated on 10 females during estrus period: only the females in heat, showing the sexual receptivity were chosen for the investigation. The experiment was performed in March - April when the air-temperature was +4 to +10ºC. The measurements were taken with the Diagnoscope EAP 871 constructed in the Institute of Biocybernetics and Biomedical Engineering from Polish Academy of Sciences in Warsaw.
The measurements of electrical conductivity were taken at the following parameters: direct current 6V and the intensity of the short-circuit current 200 µA. Electrical conductivity in the chosen points is presented conventionally in the units of electric current intensity (µA – 1 x 10^{-6}A).
The passive electrode of the diagnoscope was fixed to the wetted ear of the female fox with physiologic salt solution and the measurement points were localized by an active electrode. The active electrode was adapted to the skin at a right angle and the measurements were taken with a slight steady pressure. The time of the measurement was regulated automatically and the apparatus disconnected itself after 3 seconds.

Table 1. Localization and description of acupuncture points chosen for the experiment

Acupuncture points	Localization	Description
B_{22}	between 1-2 transverse processes of the lumbar vertebra	stimulated in cases of estrous disturbances
B_{23}	between 2-3 transverse processes of the lumbar vertebra	stimulated in cases of estrous disturbances
B_{25}	between 4-5 transverse processes of the lumbar vertebra	stimulated in cases of parturition and postpartum period disturbances
B_{31}	openings on the sacral bone from the dorsal side	stimulated during the parturition and postpartum period
B_{32}	openings on the sacral bone from the dorsal side	stimulated during the parturition and postpartum period
Lg_2	between the sacral bone and the first coccygeal vertebra /along the back/	stimulated during the parturition and postpartum period
Lg_3	between the sacral bone and the last lumbar vertebra /along the back/	stimulated during the parturition and postpartum period
Lg_4	between 2-3 spinous processes of the lumbar vertebra /along the back/	stimulated during the parturition and postpartum period
Li_4	between the thumb and the metacarpus	stimulation of that point affects the immune system
Li_{11}	on the anterolateral surface of the forearm in the depression above the radial bone epiphysis	stimulation of that point affects the immune system

10 acupuncture points: 8 stimulated in cases of estrus, parturition and postpartum disturbances (B_{22}, B_{23}, B_{25}, B_{31}, B_{32}, Lg_2, Lg_3, Lg_4) (Kothbauer & Meng, 1983) and 2 affecting immune system (LI_4 and LI_{11}), (Sciesinski, 1988, Sciesinski, 1996) were chosen for the experiment. The localization and description of chosen points are presented in the Table 1. For statistical analyses, a 2-sample t-test was used to test differences between females in estrus and diestrus periods.

Results and discussion

The results of the skin electrical conductivity (µA) in the chosen acupuncture points stimulated in cases of estrus, parturition and postpartum disturbances are presented in Table 2.

The electric potential in all checked points lying on urinary bladder meridian (B_{22}, B_{23}, B_{25}, B_{31}, B_{32}) and on the main back regulator (Lg_2, Lg_3, Lg_4) was much higher ($p<0.01$) during estrus than during diestrus. Higher potential suggests that mentioned points at that time are more sensitive for any impulse. The acupuncture stimulation of chosen points, as described by other authors (Westermayer 1979, Kothbauer & Meng 1983) can improve reproduction results.

The results of skin electrical conductivity points responsible for immune system activity in polar fox females are presented in Table 3.

There were no statistical differences in electrical conductivity in acupuncture points Li_4 and Li_{11} situated on the large intestine meridian located on forearm, between diestrus and estrus periods, even the conductivity measured during estrus appeared higher. As it was described in literature (Sciesinski, 1988; Sciesinski, 1996), stimulation of these points affects immune system. When the animals are healthy and in good condition, the electrical conductivity of specific points should not differ statistically (Sciesinski, 1996).

The presented results illustrate the differences in the skin electrical conductivity between female polar foxes in the diestrus period and those being in heat. The higher values of the skin electrical conductivity in female polar foxes during estrus are affected by physiological processes in their organisms.

Table 2. The comparison of electrical conductivity acupuncture points (μA) affecting reproduction results during diestrus and estrus in polar fox females

Acu-puncture point	Electrical conductivity (μA, 1×10^{-6} A)			
	During diestrus		During estrus	
	x	Sd	x	Sd
B_{22}	62.1A	4.91	87.3B	3.63
B_{23}	62.7A	4.49	87.6B	5.02
B_{25}	61.7A	5.46	88.1B	4.46
B_{31}	62.8A	5.33	81.2B	3.37
B_{32}	60.4A	6.06	87.0B	4.71
Lg_2	57.8A	5.34	86.2B	4.33
Lg_3	58.9A	6.87	87.7B	3.87
Lg_4	59.1A	10.27	86.0B	5.62

A,B – difference at $p<0.01$

Table 3. The comparison of electrical conductivity (μA) points stimulated during immune system disturbances, between diestrus and estrus periods, in polar fox females

Acu-puncture point	Electrical conductivity (μA, 1×10^{-6} A)			
	During diestrus		During estrus	
	x	Sd	x	Sd
Li_4	56.4	4.50	63.5	8.08
Li_{11}	54.4	4.77	61.2	7.07

References

Hyodo M., 1979: Rydoraku treatment an objective approach to acupuncture. Osaka, Japan.

Kothbauer O., Meng A., 1983: Grundlagen der Veterinar Akupunktur Spezielle Akupunktur bei Rind, Schwein und Pferd. Verlag Welsermuhl, Wols, Austria.

Sciesinski K., 1988: Producing immune reaction in adult foxes with the help of the acupuncture method. Scientifur 12, 2: 99-104.

Sciesinski K., 1996: Measurement of skin electrical conductivity at acupuncture points in healthy and diseased polar foxes. Scientifur 20.

Westermayer E., 1979: Akupunktur bei geburt und Prolapsreposition beim Rind. *Tierztliche Praxis* 1: 9-12.

IV – 10 RP

Isolation of microsatellite markers for American mink *(Mustela vison)*

A. Farid[1], I.R. Vincent[1], B.F. Benkel[1] and K. Christensen[2]
1- *Department of Plant and Animal Sciences, Nova Scotia Agricultural College, Truro, N.S. B2N 5E3, Canada. 2- The Royal Veterinary & Agricultural University, Division of Animal Genetics, Groennegaardsvej 3, DK-1870 Frederiksberg C, Denmark.*

Abstract
The objective of this study was to isolate and characterize microsatellite markers, especially tetranucleotide repeats, for American mink. A size-selected mink genomic library was constructed, and recombinant colonies (n=2435) were screened with two pools of probes. One pool included $(AAAC)_8$, $(AAAT)_8$, $(AACC)_8$, $(ATGG)_8$ and $(AC)_{15}$, and the other pool contained $(AAAG)_8$, $(AAGG)_8$, $(AGGG)_8$, $(ATAG)_8$ and $(AG)_{15}$ oligonucleotides in equal amounts. Positively hybridized colonies were bi-directionally sequenced. Thirteen of the recombinant colonies (0.53%) contained a microsatellite. One GTTT, one GGAT, four AG and seven AC repeats were detected, which may represent the relative abundances of these repeat motifs in the mink genome. One locus could not be amplified by the polymerase chain reaction. Variability of other loci was determined by genotyping 86 unrelated mink of three color types (black, brown, pastel) and wild mink trapped in northern New Brunswick (Canada). Two of the loci were monomorphic, and the other 10 generated 2, 3, 4, 5, 6, 7, 7, 9, 10 and 11 alleles (average of 6.4). Seven of the primer sets amplified DNA of American pine marten *(Martes americana*).

Introduction
Microsatellites are markers of choice for evolutionary and conservation studies, paternity testing, assignment of individual animals to specific subpopulations (Belliveau *et al.* 1999), as well as for the construction of linkage maps which are valuable tools in animal genetic improvement. Despite the economic importance of American mink (*Mustela vison*) in North America and northern Europe, information on the mink genome, compared to most other farm animal species, is very scarce. Fewer than 100 microsatellite markers have so far been identified for mink (O'Connell *et al.,* 1996; Brusgaard *et al*., 1998 a,b,c; Davis and Strobeck,1998; Fleming *et al.,* 1999; Vincent *et al.,* 2003), and more are needed to construct a rough linkage map of the mink genome. The objective of this work was to isolate and characterize microsatellite markers, particularly tetranucleotides, for mink. Although tetranucleotides are less abundant than dinucleotides in mammalian genomes (Lander *et al.,* 2002), they can be more easily and accurately scored on gels or electropherograms as a result of low intensity of stutter bands (Urquhart *et al.,* 1995; Walsh *et al.,* 1996).

Materials and Methods
Approximately 30 µg of genomic DNA from one female black mink was digested to completion overnight with *Sau*3AI. Digested fragments were size separated on a 1% agarose gel, and fragments of 300 to 800 bp were recovered from the gel and purified by phenol extraction and ethanol precipitation (Sambrook *et al.* 1989). Size-selected fragments were ligated into *Bam*HI-digested dephosphorylated pGEM-3Z vector (Promega, Madison, WI, USA). The ligated products (2 µL) were used to transform 50 µL of maximum efficiency competent *E. coli* (JM109, Promega) and were plated out on LB/ampicillin/IPTG/X-gal media and cultured overnight. Recombinant colonies were transferred onto duplicate LB/ampicillin plates and were lifted onto Hybond-N^+ nylon membranes (Amersham, Piscataway, NJ, USA) after overnight growth. Lifted colonies were fixed on membranes by baking for 2 hours at 80°C under vacuum. Cell debris were removed by incubating membranes in 100 mL of a digestion solution (100 µg/mL proteinase K, 50 mM Tris Cl, pH 7.6, 0.1% SDS and 50 mM NaCl) at 37°C with gentle agitation for at least 6 h, and were rinsed in 100 mL of 2X SSC.
Membranes were hybridized with two pools of oligonucleotide probes using a chemiluminescence DNA detection kit (Amersham) according to the manufacturer's instructions. One pool contained 50 ng

each of $(AAAC)_8$, $(AAAT)_8$, $(AACC)_8$, $(ATGG)_8$ and $(AC)_{15}$ oligonucleotides and the other contained the same amount of $(AAAG)_8$, $(AAGG)_8$, $(AGGG)_8$, $(ATAG)_8$ and $(AG)_{15}$. The concentration of each probe was doubled when membranes were re-hybridized. Pre-hybridization and hybridization were performed at 42°C in a rotisserie hybridization oven for one and three h, respectively. Membranes were exposed to the Kodak Bio-Max MR-1 X-ray films, and positively hybridized colonies were re-plated and re-hybridized for confirmation. A few bacterial cells from each confirmed colony were directly transferred to a PCR cocktail, and the DNA insert was amplified using the T7 and SP6 universal primers (Promega) at 50°C annealing temperature. Amplified DNA inserts were bi-directionally sequenced. Sequence alignment and editing were performed using the Sequencher software (Gene Codes Corp., Ann Arbor, MI), and search for repeats was performed by the Tandem Repeat Finder program (Benson, 1999). Primers for the amplification of microsatellite loci were designed using the Oligo Primer Analysis Software, Version 6 (Molecular Biology Insight, Cascade, CO).

Following optimization, forward primers were fluorescently labeled with NED (Applied Biosystems), 6-FAM or HEX (Invitrogen, Burlington, ON). Amplifications were performed in 15.0 µL total volumes containing (final concentration) 0.1% Tween 20, 1X PCR buffer, 0.2 mM each dNTP, 800 nM each primer, 0.24 unit of *Taq* polymerase (Roche, Laval, QC) and 20 to 50 ng of genomic DNA. All loci were amplified using the 2-step PCR protocol in an Eppendorf Master Cycler (Hamburg, Germany), which was programed at 95^BC initial denaturation for 4 min, followed by 30 cycles of denaturation at 94^BC and primer-specific annealing temperature (Table 1), each for 30 s.

The Mvi804, Mvi1008, Mvi1010, Mvi1012, Mvi1014 and Mvi1017 loci yielded split peaks on electropherograms and unstable allele sizes, which were caused by the nontemplated addition of adenosine to the 3' end of the PCR products (Hu, 1993; Brownstein *et al.,* 1996; Magnuson *et al.,* 1996). Leaving PCR products of the Mvi804 and Mvi1017 at room temperature for at least two weeks, and those of Mvi1008 and Mvi1012 for at least one week, prior to genotyping, which resulted in the addition of adenosine to PCR products, resolved the problem. Leaving PCR products of the Mvi1010 and Mvi1014 for at least two weeks partially resolved the problem, and the use of TaKaRa LA *Taq* polymerase (Fisher Scientific, Ottawa, ON) improved stability of the Mvi1010 peaks.

Polymorphism at each locus was determined by genotyping 86 mink; 25 black, 20 pastel, 20 brown and 21 wild. Black mink originated from four large breeding ranches in Nova Scotia, and were unrelated to each other for at least one generation. Samples of pastel and brown mink were from large-size breeding ranches in Prince Edward Island, and wild mink were trapped in a 40 km^2 area in northern New Brunswick (Belliveau *et al.* 1999). Genotyping was performed using an ABI Prism 377 DNA sequencer equipped with the GeneScan and Genotyper software (Applied Biosystems, Inc., Foster City, CA). Diluted amplicons and a size marker (400 HD ROX, Applied Biosystems) were denatured at 94^BC for two minutes prior to loading (1.5 µL) onto the gel slot. Genotypes of all mink were determined at every locus. Observed and expected heterozygosities were computed using the Popgen software (http://www.ualberta.ca/-fyeh).

Results and discussion

Eighteen of the 2435 recombinant colonies were positively hybridized and DNA inserts were sequenced. Thirteen DNA inserts contained a microsatellite (0.53% of recombinant colonies) and five inserts had fewer than five uninterrupted dinucleotide repeats, which were excluded from further analysis. Searches of GenBank with the flanking sequences of the microsatellites confirmed that none has been previously reported. The repeat motifs and GenBank accession numbers of the loci are shown in Table 1. Although 80% of the probes were tetranucleotide-specific oligonucleotides, only two loci containing tetranucleotide repeats were revealed (one GTTT and one GGAT), while four AG and seven CA repeats were detected. The number of each type of repeat identified may reflect the relative abundance of each type of repeats in the mink genome, and are consistent with those in the human (Lander *et al.,* 2001). Excluding Mvi1017, which contained six repeats (CCT, TCC, CTT and GA), the mean numbers of the longest uninterrupted repeating units were 13.9 for AC, 11.0 for AG and 5.5 for tetranucleotides (Table 1).

Table 1. Repeat motifs and GenBank accession numbers of microsatellite loci.

Locus	Repeat motif	GenBank Accession number
Mvi804	$(GTTT)_6$	AY602193
Mvi1001	$(TC)_{11}N_{184}(TC)_5(CT)_2$	AY602194
Mvi1006	$(CA)_{16}$	AY602195
Mvi1007	$(GC)_5(CA)_{12}$	AY602196
Mvi1008	$(CA)_6(TA)_2(CA)_6TG(CA)_4CG(CA)_3N_{70}(TG)_{11}$	AY602197
Mvi1009	$(AC)_{12}$	AY602198
Mvi1010	$(GT)_4(TC)_{13}$	AY602199
Mvi1012	$(GGAT)_3GAAT(GGAT)_5$	AY602200
Mvi1013	$(AG)_9GATA(AG)_6$	AY602201
Mvi1014	$(TG)_{16}$	AY602202
Mvi1015	$(AC)_8AA(AC)_{15}$	AY602203
Mvi1016	$(GT)_{15}$	AY602204
Mvi1017	$(CCT)_3TCT(CCT)_4(TC)_2(CT)_2(TCC)_{12}N_{26}(TTC)_4(TCC)_9$ $(TTC)_3TGCT(CTT)_{12}N_{43}(GA)_6GGGCAT(GA)_7$	AY602205

The mean number of AC repeating units falls within the 13.4 to 15.1 reported by others for mink (O'Connell *et al.,* 1996; Fleming *et al.,* 1999; Vincent *at al.,* 2003).
The unique sequence flanking the repeating unit of the locus Mvi1015 was too short, resulting in the failure of the primer set to produce a specific band. Sequences of the primers for the remaining 12 microsatellites are shown in Table 2. Ten of the loci were polymorphic in the panel of 86 mink, and generated between 2 (Mvi1013) and 11 (Mvi1016) alleles. Observed heterozygosity (H_o) ranged from 0.15 (Mvi1013) to 0.84 (Mvi1016), and expected heterozygosity (H_E) ranged from 0.25 (Mvi1013) to 0.85 (Mvi1016) (Table 2). The means of number of alleles, H_o and H_E of the polymorphic loci were 6.4, 0.54 and 0.67, respectively. Although estimates of the number of alleles, H_o and H_E are the characteristics of each locus, they also reflect the diversity of the animals that were genotyped. The estimates suggest that at least six of these loci, each with six or more alleles, are very useful for population genetics studies. Tetranucleotides had a lower level of variability than dinucleotides in this study, as one (Mvi1012) was monomorphic and one (Mvi804) had only 3 alleles.

Table2. Primer sequence, annealing temperature (T_A), number of alleles, allele sizes, and observe (H_O) and expected (H_E) heterozygosities of the mink microsatellites[1]

Locus	Primer sequence (5'-3')	T_A, ^{B}C	No. of alleles	Allele size, bp	H_O	H_E
Mvi804	F: GGAAATACCTATCATGGC R: AAGAGTTGTAAGGAAGTTCCAG	59.1	3	149-157	0.43	0.57
Mvi1001[2]	F: AGTGCAAGAAGGACGTAATGTG R: AGAGACCGAGAGAGCATGTATG	59.1	1	152	0.0	0.0
Mvi1006	F: CCAAGCAGGATTCAGCCTATTC R: AAGGCCATGCACTAGGTAA	59.1	10	149-167	0.79	0.79
Mvi1007	F: TAAGAGGCTTGCCGTGTTCA R: TCAGGACTGTCTCTTCGGGATG	59.1	4	248-254	0.59	0.64
Mvi1008[3]	F: GATGGGGATAAACCTGCTAATC R: CCCCAAATGAACCTCCATACAA	59.1	5	210-218	0.59	0.68
Mvi1009	F: CAAGCCTCCACAACTGT R: ACAATGGTGCTATGTTAGTTA	62.2	6	152-162	0.41	0.64
Mvi1010	F: ATCAAGCCCCACGTCATACTCCC R: GGCAGCCGCTTCATGACTGAGACAC	66.7	7	167-181	0.63	0.71
Mvi1012	F: ACTGATGCCTGCCATAGCTC R: TACCCAGCCTGGAGTAGTAGTTTG	59.1	1	258	0.0	0.0
Mvi1013	F: GCTCCATACTTGTCCAACAACTTCC R: CTGCTTCTCCCTCTCACCCTACC	59.1	2	166-170	0.15	0.25
Mvi1014	F: TCTGCATGTAAAATATGGGATA R: TCACAGGTCCTTGCTTGAACAC	56.5	9	136-152	0.71	0.81
Mvi1016	F: CTGCTTCTCTGCCTACTTCT R: TTGTTCCCTTCCTATTATCTGT	59.1	11	218-238	0.84	0.85
Mvi1017[4]	F: TCCTCTCATGTGTCTTTGGGTTAT R: TGCTCTTCAGGGAGTCTGCTTCT	66.0	7	326-349	0.28	0.76

1- Optimum $MgCl_2$ concentration is1.5 mM for all the primer sets (supplied in the PCR buffer), except for the Mvi1009, which requires 1.0 mM additional $MgCl_2$.
2-The first repeat of Mvi1001 is too close to the 5' end of the sequence, and is not included in the amplified segment.
3- Amplified segment contains both repeats of the Mvi1008.
4- Amplified segment contains all repeats of the Mvi1017

Seven of the primer sets (58%) generated specific PCR products in American pine marten (*Martes americana*). Two loci were monomorphic, and five loci generated between two and five alleles in six related individuals (Table 3). Interestingly, the Mvi1012, which was monomorphic in mink, generated two alleles in pine martens. Amplification of pine marten DNA with primers designed for mink, which belong to the same subfamily *(Mustelinae),* has been reported by O'Connell *et al.* (1996), Vincent *at al.* (2003), and Fleming *et al.* (1999). Likewise, 3 of the 13 microsatellites developed for American marten amplified mink DNA (Davis and Strobeck, 1998). The cross-species amplification of microsatellite primers is a fast and inexpensive method of developing genetic markers in the members of *Mustelidae* family.

Table 3. Annealing temperature (T_A), number of alleles and allele sizes of mink primers that amplified DNA of American pine marten[1]

Locus	T_A,°C	Number of alleles[2]	Allele size (bp)
Mvi804	51.4	1	153
Mvi1001	59.4	1	151
Mvi1006	65.0	4	156, 162, 164, 166
Mvi1007	56.5	5	259, 261, 263, 265, 267
Mvi1008	59.4	4	215, 219, 223, 227
Mvi1012	51.4	2	270,274
Mvi1014	56.5	2	118,122

1- Optimum $MgCl_2$ concentration is1.5 mM for all the primer.
2- In six related individuals.

Acknowledgments
We gratefully acknowledge the financial contribution of Canadian Mink Breeders Association, Nova Scotia Fur Institute and Nova Scotia Department of Agriculture and Fisheries (Agri-Focus 2000 program). Technical assistance of Tannille Crossman is greatly appreciated.

References

Belliveau, A.M., Farid, A., O'Connell, M. and Wright, J.M. 1999. Assessment of genetic variability in captive and wild American mink (*Mustela vison*) using microsatellite markers. Can. J. Anim. Sci. 79: 7-16.

Benson, G. 1999. Tandem repeat finder: a program to analyze DNA sequences. Nucl. Acids Res. 27:573-580.

Brownstein, M.J., Carpten, D. and Smith, J.R. 1996. Modulation of non-templated nucleotide addition by *Taq* DNA polymerase: Primer modification that facilitate genotyping. Biotechniques 20:1004-1010.

Brusgaard, K., Shukri, N., Malchenko, S.N., Lohi, O., Christensen, K. and Kruse, T. 1998a. Three polymorphic mink, *Mustela vison*, dinucleotide repeats. Anim. Genet. 29: 153

Brusgaard, K., Malchenko, S.N., Christensen, K., Lohi, O. and Kruse, T. 1998b. A polymorphicmink *(Mustela vison)* dinucleotide repeat. Anim. Genet. 29: 467

Brusgaard, K., Holm, L-E., and Lohi, O. 1998c. Two polymorphic mink *(Mustela vison)* dinucleotide repeat loci. Anim. Genet. 29: 468.

Davis, C.S. and Strobeck, C. 1998. Isolation, variability, and cross-species amplification of polymorphic microsatellite loci in the family Mustelidae. Mol. Ecol. 7:1776-1778.

Fleming, M.A., Ostrander, E.A. and Cook, J.A. 1999. Microsatellite markers for American mink *(Mustela vison)* and eramine (*Mustela erminea*). Mol. Ecol. 8: 1351-1362.

Hu, G. 1993. DNA polymerase-catalyzed addition of nontemplated extra nucleotides to the 3' end of a DNA fragment. DNA and Cell. Biol. 12:763-770.

Lander, M.L. *et al.,* 2001. Initial sequencing and analysis of the human genome. Nature, 409: 860-921.

Magnuson, V.L., Ally, D.S., Nylund, S.J., Karanjawala, Z.E., Rayman, J.B., Knapp, J.I., Lowe,A.L., Ghosh, S. and Collins, F.S. 1996. Substrate nucleotide-determined non-templated addition of adenine by *Taq* DNA polymearse: Implications for PCR-based genotyping and cloning. Biotechniques 21:700-709.

O'Connell, M., Wright, J.M. and Farid, A. 1996. Development of PCR primers for nine polymorphic American mink *Mustela vison* microsatellite loci. Mol. Ecol. 5: 311-312.

Sambrook, J., Fritsch, E.F. and Maniatus, T. 1989. *Molecular cloning: a laboratory manual*. 2nd edn. Cold Spring Harbor Laboratory Press, NY, USA.

Urquhart A., Oldroyd, N.J., Kimpton, C.P., and Gill, P. 1995. Highly discriminating heptaplex short tandem repeat PCR system for forensic identification. Biotechniques 18:116-121.

Vincent, I.R., Farid, A. and Otieno, C.J. 2003. Variability of thirteen microsatellite markers in American mink *(Mustela vison*). Can. J. Anim. Sci. Vol. 83: 597-599.

Walsh, P.S., Fildes, N.J. and Reynolds, R. 1996. Sequence analysis and characterization of stutter products at the tetranucleotide repeat locus vWA. Nucl. Acid Res. 24:2807-2812.

IV – 11 RP

Litter size, weaning success, and nursing mortality in chinchillas (*Chinchilla lanigera*) in relation to cage illumination

Lidia Felska[1], Marian Brzozowski[2]

[1] Department of Ruminant Animal Science, Laboratory of Fur Animals, Agricultural University of Szczecin, ul. Judyma 10, 71-450 Szczecin, e-mail: l.felska@biot.ar.szczecin.pl

[2]Division of Fur and Pet Animals, Warsaw Agricultural University, ul. Nowoursynowska 166, 02-787 Warszawa, Poland

Abstract

The aim of this study was determine effects of light intensity on litter size, number of weaned per litter and mortality rate during nursing. Study was performed on a reproduction farm in western Poland, during1999-2003. The analysis covered reproduction performance of 250 females of the standard variety. Light intensity was measured with a photoelectric light meter LS-200 and ranged between 0 and 270 lx. The chinchillas were assigned to 9 groups, 30-lx interval each. No statistical differences were found between the groups in relation to light intensity. Both litter sizes and number of weaned per litter grew along with increasing light intensity. The lowest mortality was found at the highest light level, i.e. 241-270 lx (group IX) – 4.17%. Nursing mortality showed a falling trend with growth in cage illumination level. The range between 241 and 270 lx was the optimal range of light intensity in this study.

Introduction

Wild chinchilla is a nocturnal species inhabiting rock cracks and hollows of mountain slopes of the Andes. The species, however, has been observed to live also a diurnal life in its natural habitat [Hoefer, 1994; Mohlis, 1983; Walker, 1975]. Barabasz [2003] also found that farmed chinchillas exhibit, as a result of domestication, increased activity during morning hours and during the day.

Reproduction of chinchillas and other farmed animals alike depends heavily on climatic conditions and, in the case of indoor housing, on the microclimate of the sheds, primarily on temperature, humidity, light, and feeding [Bernard et al. 1999]. Effect of light on reproductive processes consists in regulation of gonad activity. Growth of the ovarian follicles is controlled by folitropin, or the follicle stimulating hormone (FSH), whose synthesis and secretion from the anterior pituitary is stimulated by gonadoliberin (FSH/LH-RH), a decapeptide hormone released by the hypothalamus. Information on the amount of hypothalamus-released hormones is conveyed via thermal and light stimuli [Turner and Bagnara, 1978].

Chinchilla sheds should be dry, well illuminated, well ventilated, without draughts, and free of fungi [Barabasz, 1996]. The optimal temperature should remain within the range 16-22°C, relative humidity 50-70%, and air flow between 0.2 and 0.3 m/s [Barabasz, 1996; Felska, 1999; Parker, 1982]. Little has been reported, however, on the light conditions that are optimal for chinchillas. At present, artificial illumination is becoming more and more common in chinchilla sheds, and no adverse changes in the organisms of the animals have been found that would be a consequence of lack of sunlight. Artificial illumination depends on the number, type, power, and distribution of lamps. Usually, incandescent lamps of 60, 75, or 100 W, as well as fluorescent lamps of 25-40 W are used to illuminate the sheds; mercury or sodium vapour lamps are rare. The colour of artificial light must resemble the spectrum of natural light.

Due to chinchilla reproduction specificity, i.e. their low fertility in terms of number of litters and their sizes [Gromadzka-Ostrowska et al., 1985], intensive research has been in progress to improve reproduction parameters, often through enhancement of microclimate of the sheds, also through finding optimal light illumination.

The aim of the study was to determine the effect of light intensity on litter size, weaning success in terms of the number of weaned young, and pre-weaning mortality rate in chinchillas.

Material and Methods

The studies were performed on Alex Chinchilla Farm in Nowogard, one of the largest chinchilla breeding farms in Poland, during 1999-2003. Chinchillas on this farm are housed only indoors, in polygamous breeding system cages arranged in four-level sets. A polygamous set is composed of four females and a single male. Reproduction performance of 250 standard chinchilla females was evaluated. The females were at ages 2-5 years, i.e. in

the most fertile period of life. The first litters of the females were excluded from the analyses.

The chinchillas were managed in a shed with controlled constant temperature and relative humidity. In order to evaluate the microclimate of the shed, the following measurements were done: air temperature and humidity, air evaporative cooling, air flow, and light intensity. The temperature remained within 18-20°C, relative humidity within 50-60%, air evaporative cooling was 12.4-16.7 mW/cm^2, while air flow was 0.2 m/s.

The shed lacked windows; artificial light, produced by 40-W fluorescent lamps, was the only source of illumination. In 1998, a 12-hour light regime was introduced on Alex Chinchilla Farm, with average illumination intensities being about 5 W/m^2. In the beginning of 2001, in a selected part of the shed, 5 new lamps of 116 W were mounted. The average illumination of this part was 10 W/m^2.

Light intensity was measured inside the chinchilla cages by means of a photoelectric light meter LS-200 (Sonopan, Poland). Light was measured along the six planes: right, left, front, hind, up and down, and the mean for a particular cage was calculated from these six measurements. Light intensities in the cages ranged between 0 and 270 lx. The animals were distributed into nine 30-lx groups. The number of chinchillas in each illumination group and the ranges of light intensity is presented in Table 1.

Table 1. Number of females in each illumination group

Group	Light intensity [lx]	Number of females
I	0-30	62
II	31-60	37
III	61-90	28
IV	91-120	29
V	121-150	31
VI	151-180	16
VII	181-210	18
VIII	211-240	19
IX	241-270	10
Total	0-270	250

In each group, the number of offspring born and weaned from the litter and death rate during maternal nursing were analysed. The data were then computed and statistically processed using a spreadsheet and Statistica 6.0 software package. The following descriptive statistics were calculated: arithmetic mean (M), standard deviation (SD), and coefficient of variability (CV). The non-parametric Kruskal-Wallis test was applied for testing significance of differences, since the variables were of ranked character and were not normally distributed.

Results

Table 2 presents statistical characteristics of mean litter size and number of weaned young per litter.

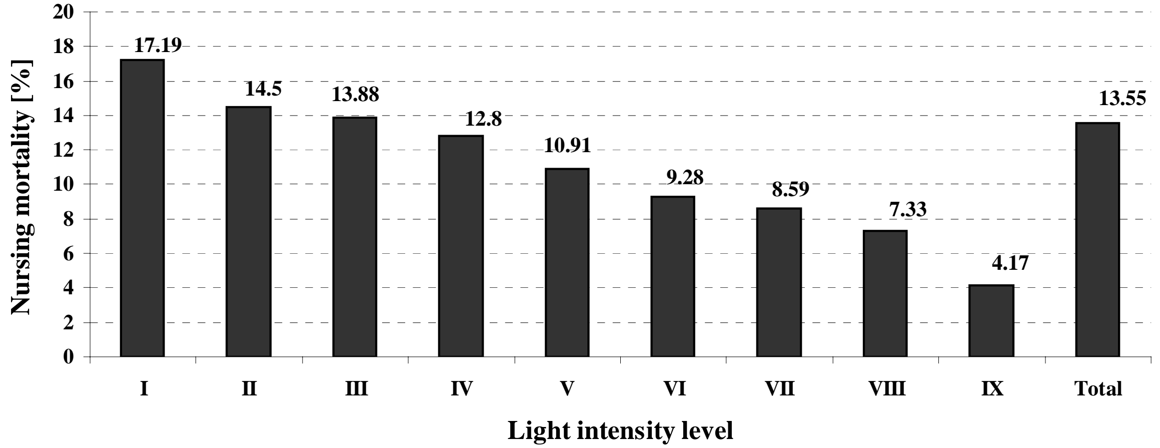
The highest numbers of both born and weaned offspring per one litter were recorded for the highest light intensities, i.e. at 241-270 lx (group IX), 2.25 and 2.17 young respectively. In contrast, the lowest numbers of born and weaned per one litter were recorded in the group of 0-30 lx (group I) and 211-240 lx (group VIII), respectively 2.03 and 1.84 (born) and 1.72 (weaned). The differences, however, proved statistically non-significant. The largest litters and the best weaning rates were achieved under light intensities ranging between 181 and 270 lx.

Figure 1 depicts the mortality of young during maternal nursing. The testing did not yield any statistically significant differences in deaths during nursing between the illumination groups; however, the highest mortality rate was found under the lowest light intensities, 0-30 lx (group I), 17.19%, while to lowest was found under the highest light intensities, 241-270 lx (group IX), 4.17%. The overall nursing mortality was about 13.5%. Despite no significant differences, the light intensity apparently influenced the pattern of nursing mortality; the mortality dropped with increasing illumination intensities.

Table 2. Statistical characteristics of chinchilla births and weaning

Group	Born chinchillas per litter			Weaned chinchillas per litter		
	M	SD	CV	M	SD	CV
I	2.03	0.79	38.92	1.72	0.91	53.18
II	2.15	0.73	34.10	1.83	0.84	45.74
III	2.08	0.82	39.36	1.76	0.77	43.64
IV	2.18	0.80	36.71	1.85	0.78	43.47
V	2.18	0.69	31.69	1.90	0.71	37.70
VI	2.20	0.79	36.05	1.98	0.82	41.52
VII	2.21	0.74	33.44	2.06	0.90	43.64
VIII	1.84	0.47	25.68	1.72	0.54	31.49
IX	2.25	0.61	27.02	2.17	0.76	35.14
Total	2.16	0.74	34.20	1.95	0.77	39.64

Figure 1. Nursing mortality in each illumination group



Discussion

Chinchillas belong to polyoestrous animals, i.e. having more than one sexual cycle in one year. The number of produced pelts, and thus the profit of the breeder, depends on the number of born and weaned chinchillas. The chinchillas seem to have a large reserve of unused reproductive potential. An adult female produced about 16 ovarian follicles in one sexual cycle, of which only 4 maturate during the oestrous stage of the cycle and, hence, given environmental and genetic conditions are good, four young per litter should be achieved [Barabasz, 2001]. Puzder and Novikmec [1992] have stated that the number of maturating egg cells per cycle ranges between 4 and 16, and the annual number of born offspring is 4-8. According to Socha et al. [2001a, 2001b], litter size may range from 1 to 5. Most often, however, 2 young are born in a litter [Barabasz, 1997; Puzder and Nowikmec, 1992; Socha et al., 2001a; Sulik, 1994]. This has been confirmed in this study. Lanszki et al. [1998] obtained 2.04 born and 1.84 weaned young chinchillas.

Our results correspond to those reported by Garcia et al. [1989], who stated that light influences chinchilla reproduction, since females kept in better-illuminated cages gave births to larger litters compared to those managed in the cages with lower light intensity. According to Garcia et al. [1989], the highest mortality rate is during the first two weeks of life and reaches as high as 20%. Such high mortality may result from insufficient milk supply by dams as well as from low tolerance of the young to lower temperatures, below 10°C. Lanszki [1996] observed that the highest mortality occurs during the first week of postnatal life and reaches about

15.3%. Felska et al. [2002] states, however, that pre-weaning death rate ranges between 10.4% to 17.1%, which corresponds to the results of this study. It has been found in this study that higher light intensities have a positive effect on raising young chinchillas. These results correspond to those published by Garcia et al. [1989], who observed that pre-weaning mortality in the cages placed at the 4th level, where light intensity was higher than at lower levels of cages, was lower compared to that recorded at the 3rd and 2nd levels of cages.

The elevated intensity of illumination applied in our experiment resulted in higher numbers of born and weaned offspring per litter as well as in lower death rate of young chinchillas during their maternal nursing period. The range of light intensity between 241 and 270 lx represented the optimal range in this study.

Acknowledgements

The project was financed by State Committee Scientific Research, No: KBN-3PO6Z 02623

References

Barabasz B., 1996: Zoohygiene in breeding practice. [In Polish]. Biul. Inf. Hod. Szynszyli 4, 14-16.

Barabasz B., 1997: Chinchillas in their natural environment. [In Polish]. Biul. Inf. Hod. Szynszyli 1, 17-19.

Barabasz B., 2001: Chinchillas: Breeding and Management. [In Polish]. PWRiL, Warszawa.

Barabasz B., 2003: Characterisation of traits that demonstrate domestication of chinchilla (*Chinchilla lanigera*). [In Polish]. Annales Universtitatis Mariae Curie-Skłodowska, Lublin- Polonia, vol. XXI, 2(63), Sectio EE, 71-77.

Bernard D. J., Abuav-Nussbaum R., Horton T. H., Tyrek F. W., 1999: Photoperiodic effects on gonadotropin-releasing hormone (GnRH) content and the GnRH-immunoreactive neuronal system of male Siberian hamsters. Biology of Reproduction 60, 272-276.

Felska L., 1999: Zoohygienic conditions on a chinchilla farm. [In Polish]. Prz. Hod. 7, 24-26.

Felska L., Brzozowski M., Rzewucka E., 2002: Results of chinchilla reproduction in relation to cage level and light intensity. [In Polish]. Zesz. Nauk. Prz. Hod. 64, 97-102.

Garcia X., Neira R., Schen R., 1989: Environmental effects on reproductive traits in confined chinchillas (*Chinchilla laniger* Gray). Avances en Production Animal 14 (1-2), 121-127.

Gromadzka-Ostrowska J., Zalewska B., Szylarska-Góźdź E., 1985: Peripheral plasma progesterone concentration and hematological indices during normal pregnancy of chinchillas (*Chinchilla laniger,* M.). Comp. Biochem. Physiol. 82A (3), 661-665.

Hoefer H. L., 1994: Chinchillas. Veterinary clinics of North America: Small Animal Practice. Exotic Pet Medicine II, vol. 24 (1), 102-111.

Lanszki J., 1996: The effect of ltter size and individual weight at birth on the growth and mortality of chinchillas. Scientifur 1, 42-46.

Lanszki J., Jauk E., Bognár Z., 1998: Examination of traits related to prolificacy and suckling ability in chinchillas (*Chinchilla laniger*). Scientifur 22 (3), 219-223.

Mohlis C., 1983: Información preliminary sobre la conservación y manejo de la chinchilla Silvestre en Chile. Boletín Técnico, No. 3, Corporatión Nacional Forestal, Santiago.

Parker W., 1982: Modern Chinchilla fur Farming. Barden Publishing c.o. California.

Puzder M., Novikmec J., 1992: The principal reproductive indices in *chinchilla laniger*. Veterinarstvi 42 (7), 258-259.

Socha S., Jężewska G., Gontarz A., 2001a: Quantitative chracterisation of chinchillas (*Chinchilla velligera* M.) litters. Deutsche Veterinärmedizinische Gesellschaft e. V., 12th Symposium on Hausing and Diseases of Rabbits Fubearing Animals and Pet Animals, Celle 9-10 Mai, 231-235.

Socha S., Maćkowiak I., Jeżewska G., Gontarz A., Dąbrowska D., 2001b: Analysis of standard and Polish beige chinchilla (*Chinchilla velligera* M.) fertility in selected farms. [In Polish]. Zesz. Nauk. Prz. Hod. 58, 39-46.

Sulik M., 1994: Age of first whelping in Polish and Danish chinchilla females. [In Polish]. Zesz. Nauk. Prz. Hod. 15, 185-191.

Turner C. D., Bagnara J. T., 1978: General Endocrinology. [In Polish]. PWRiL, Warszawa.

Walker E. P., 1975: Mammals of the World. Vol. 2, III ed. John Hopkins Press, 1029-1032, Baltimore.

IV – 12 RP

Evaluation of pastel fox breeding results in poland - production traits

Jakubczak A, Jeżewska G
Agricultural University of Lublin ul. Akademicka 13, 20-950 Lublin, Poland

Abstract
Material for study was females of common fox different color varieties maintained in 1978-1997, from which 4155 progenies with pastel fur were observed.
In order to estimate the efficiency of selection, evaluation of genetic and phenotypic trends were applied. Phenotypic trends were estimated as changes of phenotypic trait mean values in time. The basis for genetic trends estimation in population studied was the solution for the birth year of an individual describing the changes of genetical quality in time. Calculations were made using software BLUPf90, applying multi-trait animal model.
Selection differences for reproduction and conformation traits taken into account during selection were calculated to estimate the intensity of the process. They were accepted as differences between mean phenotypic value of a trait among young foxes chosen to general herd and mean phenotypic value of a trait for all young.
On a base of results achieved, the conclusion was drawn that positive values of genetic trends for conformation traits and number of reared animals testify to proper direction of breeding work. However, their low values point out to low efficiency of selection. This can be a result of large number of traits considered during selection. For all investigated traits with exception of litter size at birth, an increasing tendency was found during the years under investigation which proves, the breeding work was conducted properly in this herd.

Introduction
The beginnings of original Polish pastel fox breeding are in 1972 when a female of silver hair in one of Poznań farms born a litter consisting of black-silver (so-called “standard”) and beige animals. The female along with the litter was purchased by ZHZF in Jeziory Wielkie [4, 6, 8]. This mutant was initially called “pearl of lakes” and then pastel fox as an analogy to coypu and pastel mink.
In the first years of pastel fox breeding (1972-1975), the general goal was to reproduce brown animals as soon as possible. It had to be done by reproduction of the mutated gene. Therefore, animals were mated related to one another. It led to the increase of inbreeding due to small number of individuals and common primary origin from the same foxes. Inbred depression that caused negative biological effects was a result. This variety of animals was characterized with hyperexcitability (timidness, aggressiveness, abnormal mobility) and brown-colored females damaged their litter more often than others. Also cases of submaxilla breaking due to strong squeezing the jaws on forks for animal catching were observed, which proved both great irritability and the fragility of their bones [4, 5, 6]. The problem that arose was to determine whether the symptoms are associated with new color genes (e.g. due to pleiotropic action) or are they a result of much advanced inbreeding. The thesis was drawn that reproduction and breeding problems did not result from unfavorable interaction of pastel color gene, but they were effect of strong inbreeding of animals. It appeared that inbreeding at foxes easily invoke inbred depression, which is usually manifested with the decrease of animal viability, condition worsening and decrease of fertility.
Janusz Maciejowski began organized breeding upon new mutation variety in 1976 [6]. The herd consisted of 13 pastel males and 9 females. Moreover, some silver foxes were vectors of brown color genes. Analysis of these animals’ origin revealed profound inbreeding, because rapid reproduction of mutated gene was the initial aim of breeding. Decision of suspension of the pastel fox mating among themselves was a remedy.
At the first stage (1976-1980), intensive reproduction of new color variety genes was performed through mating the pastel with silver foxes and at the same time, avoiding of mating among themselves due to the threat of inbreeding effects [8]. No selection among pastel variety was made except from the culling of some animals because of their bad health in this period [4]. At the same time, mating according to the following scheme was performed:

♀ silver × ♂ pastel
♀ pastel × ♂ ½ pastel*
♀ ½ pastel* × ♂ pastel
♀ ½ pastel* × ♂ ½ pastel*

** ½ pastel – common fox of different color varieties, vectors of pastel gene to a minimum extent related with partners*

Mating of pastel males with females – vectors of pastel gene as well as reciprocal mating: males – vectors of pastel gene with pastel females, was the most preferred.
Pastel foxes were crossbred among themselves again in 1980 when the herd consisted of 55 females and 69 males on a basis of individually prepared mating schemes in which animal relation was taken into account.
Since 1981, selection among animals with pastel hair has been conducted in order to achieve foxes with positive fur traits. It can be concluded that general directions of selection had to be as follows: color type (the most desired shade), structure of hair cover, body structure traits, fertility, prolificacy, maternal solicitude and soft temper.
Elaboration of the structure assessment standard for pastel fox by Maciejowski, Sławoń and Dąbrowska in 1984 [9] was an important moment in new variety breeding. Despite changes introduced, directions of the color variety improvement were not changed. Besides commonly accepted fur traits (density, length of hair, hair uniformity, elasticity, silkiness), dark brown animals of blue shade were accepted as the most desired type, because (apart from aesthetic virtues) animals with darker fur show much less susceptibility to become turned red and faded color as compared to light brown individuals [3, 4].
The present paper is aimed to evaluate the results of breeding upon pastel fox in 1978-1997 through estimation the selection differences as well as genetic and phenotypic trends of some performance traits.

Methods
Material for study was originated from fur animal farm in Jeziory Wielkie near Poznań. Females of common fox of different color varieties maintained in 1978-1997, from which 4155 progeny with pastel fur were observed. On a base of breeding documents, data from reproductive performance of general herd animals as well as results of young animals rearing and structure assessment were collected. Young pastel foxes were assessed after achieving full maturity of hair cover in a context of their conformation every year [9]. In total, 1066 litters, in which at least one individual of pastel color from each mating occurred, were taken into account.
Reproduction and conformation traits covariance components were estimated by means of the REML method based on a multitrait animal model using VCE 4.2.5 computer programme by Eildert Groneveld [2].
In order to estimate the efficiency of breeding, evaluations of genetic and phenotypic trends were applied. Phenotypic trends were estimated as changes of phenotypic trait mean values in time. The basis for genetic trends estimation in population studied was the solution for the birth year of an individual describing the changes of genetic quality in time. Calculations were made using software BLUPf90 by Ignacy Misztal [7], according to following model:

- in a case of conformation traits

$$\mathbf{y_{ijklm} = \mu + R_i + PW_j + RS_k + a_l + e_{ijklm}}$$

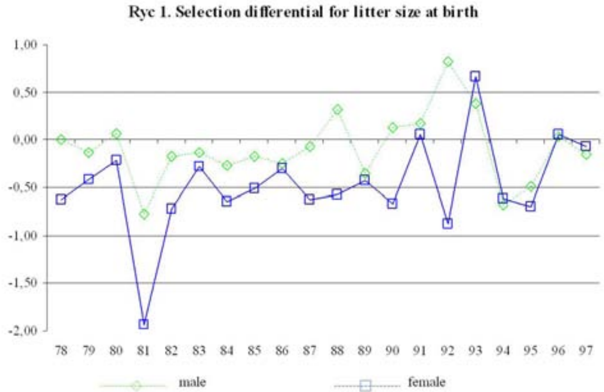
where: y_{ijklm} – vector of traits analyzed
μ - mean value of traits for population
R_i – constant effect of birth year
PW_j – constant effect of interaction sex * mother’s age
RS_k – constant effect of interaction birth year * whelping season
a_l – random effect of an individual
e_{ijklm} – random error

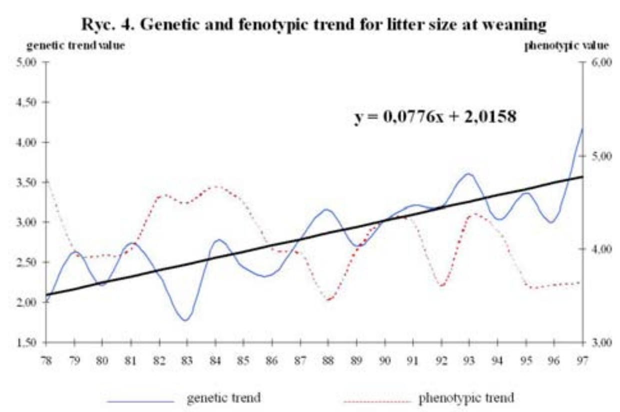
- for reproduction traits

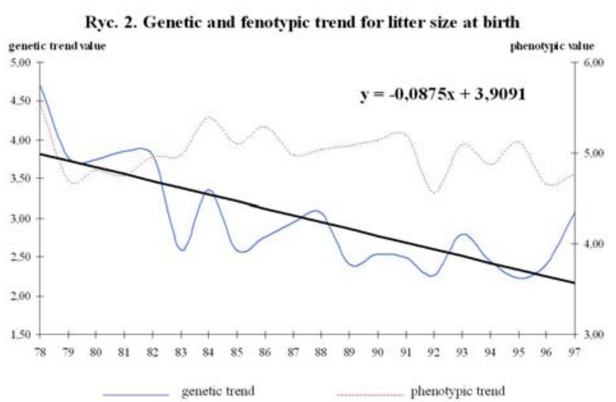
$$\mathbf{y_{ijklm} = \mu + R_i + W_j + RS_k + a_l + p_l + e_{ijklm}}$$

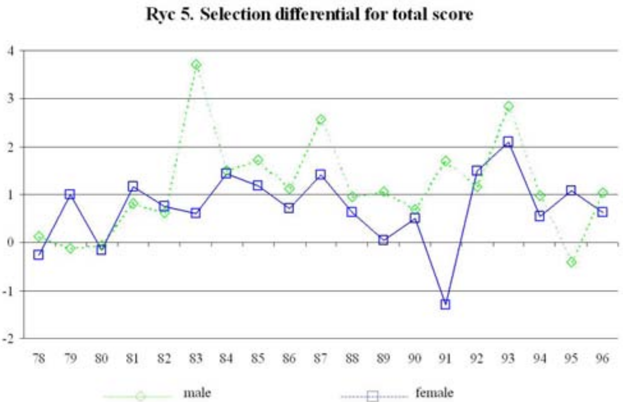
where: y_{ijklm} – vector of traits analyzed
μ - mean value of traits for population
R_i – constant effect of birth year
W_j – constant effect of animal’s age
RS_k – constant effect of interaction performance year * whelping season
a_l – random effect of an individual
p_l – random effect of constant environment for a given animal
e_{ijklm} – random error

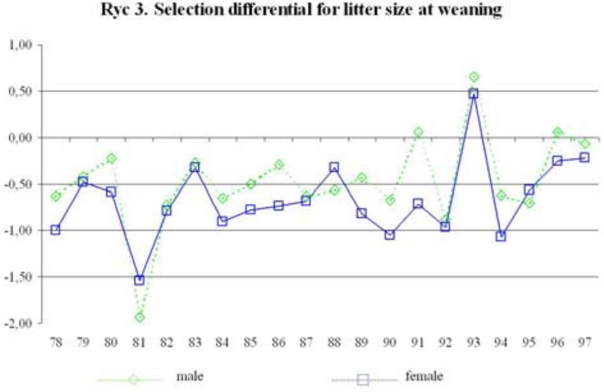
Selection differences for reproduction and conformation traits taken into account during selection were calculated to estimate the intensity of the process.

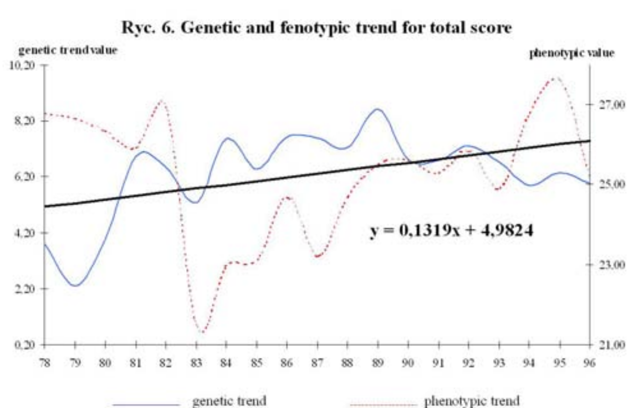












They were accepted as differences between mean phenotypic value of a trait among young foxes chosen from the general herd and mean phenotypic value of a trait for all young animals (all animals that achieved fur maturity were subjected to assessment every year). Selection was achieved using the method proposed by Maciejowski and Jeżewska [5].

Results

Table1 presents the statistical characteristic of the traits (means and coefficients of variability) regarding the year of evaluation. In order to properly characterize and assess breeding performed in 1978-1997, it was necessary to evaluate selection differences and to calculate genetic and phenotypic trends for all traits taken into account during selection. Calculated selection differences and

trends for number of born and reared young foxes as well as for total conformation assessment are presented on Figures 1 - 6. Selection differences for number of animals born archieved the most favorable values between 1988 and 1993. Over the entire period, they were higher for males The lowest values were recorded in 1981 corresponding to the beginning of mating of mainly pastel animals among themselves. It can be observed that selection carried out for that trait did not give sufficient effects, because in the first years of the period individuals of high prolificacy were mated, and at negative selection differences there was no opportunity to achieve positive trend. However, the phenotypic trend persisted at constant level, which results from the improvement of environmental conditions (Figure 2). Figure 3 presents selection differences for number of reared young foxes. The lowest values were recorded in 1981 and positive values were observed only in 1991 for males and in 1993 for both sexes. During all period in question, selection differences for males exceeded values for females, which was consistent with commonly accepted rule that more intense selection is applied to males because of higher genetic potential of interaction with population. Maciejowski and Jeżewska presented similar method of selection difference calculation. Despite of lower selection differences, a positive genetic trend was achieved, which can be the result of environmental condition improvement and decreasing the level of herd inbreeding. However, in spite of positive genetic trend, no significant improvement of number of reared young foxes was achieved (Figure 4).

Table 1. Characteristic of vixens’ reproduction and total number of scores indices in subsequent years

Year	Body conformation total scores			Female’s reproduction traits				
					Mean litter size per female at birth		Mean litter size per female at weaning	
	n	**X**	**CV%**	**n**	**X**	**CV%**	**X**	**CV%**
1978	40	26.77	6.61	87	3.83	29.56	2.10	34.31
1979	91	26.67	6.90	146	3.08	31.85	1.62	32.56
1980	112	26.35	6.30	174	3.59	38.89	1.94	43.97
1981	127	25.92	7.91	204	2.56	37.59	1.76	40.36
1982	213	26.95	5.34	159	3.36	33.93	2.81	35.73
1983	243	21.44	14.13	175	4.03	29.66	3.15	33.91
1984	277	22.97	9.97	325	3.86	31.13	2.82	39.15
1985	473	23.12	10.03	283	3.80	32.48	2.92	35.35
1986	496	24.69	7.86	274	4.05	30.29	2.52	39.48
1987	496	23.22	8.23	273	3.76	30.51	2.87	38.63
1988	134	24.69	7.61	274	2.64	32.15	1.30	40.53
1989	447	25.51	5.49	272	3.88	29.52	2.66	39.81
1990	152	25.64	4.95	249	3.96	30.32	2.70	37.31
1991	80	25.3	6.28	190	4.33	28.89	2.58	41.10
1992	45	25.84	6.70	182	3.12	33.23	1.43	43.48
1993	154	24.91	8.11	115	4.42	30.72	2.97	40.53
1994	60	26.78	6.05	103	3.98	32.24	2.82	39.03
1995	84	27.58	4.02	117	3.61	33.33	1.78	45.13
1996	131	25.16	8.43	144	3.28	31.92	1.98	39.76
1997	-	-	-	157	3.21	33.37	2.15	41.76
Total	3855	26.67	7.42	3903	3.62	32.08	2.34	39.09

In a case of conformation assessment, changes are the best observed on a base of total evaluation. During all period studied, selection differences were positive. The only exception is 1991 when cardiac-pulmonary syndrome occurred on farm as well as 1995 (Figure 5). The highest values were recorded in 1983, 1987 and 1993. Thus, results plotted on Figure 6 presenting positive values of genetic and phenotypic trends do not differ greatly.

Selection differences and trends estimated in present paper for number of born and reared young foxes are difficult to compare, because there is few data in literature addressing that problem. Usually, mean numbers of born and reared young foxes in following years are given [1]. Negative selection differences for prolificacy were due to the fact that all young foxes of pastel color were incorporated to the herd regardless from how large litters from which they originated. The main selection stress was made on conformation traits. Negative selection differences for body size during period studied can be accounted for different factors. First of all, when determining the general herd every year, the best animals regarding to hair cover quality were chosen (animal’s size was the secondary factor). There is a conviction among fox breeders that an animal achieves its real size at the second year of life. Another reason of negative selection differences was fact that all progeny that achieved fur maturity were retained for further breeding. Frequent observations made on farm in Jeziory Wielkie allowed the conclusion that pastel fox is characterized with slightly smaller body size as compared to silver or flame fox. It is perhaps caused by their curious variety or the fact that the level of the trait was neglected for many years of breeding.

Discussion

The first pastel foxes that were reproduced on farm were characterized with light brown color with slight red shade. Since the beginning of breeding, achievement of animals with dark brown hair cover with blue shade has been the goal. Selection pressure on that color type was intense and only young foxes with structure traits similar to standard were incorporated to general herd. Visible difference refers not only to color intensity of particular individuals, but also number of color animals similar to standard. The fact that almost all population of pastel foxes has hair cover color similar to standard (i.e. brown with blue shade) is the effect of such great selection stress. At the beginning of breeding, number of animals with color similar to standard was very low. The effect was achieved not only due to darkening of cover hair, but also lowering the level of cover silvering.

The crisis of fur market and changes in worldwide fashion significantly inhibited development of the color variety breeding. Nevertheless, breeding conducted for many years allowed achieving animals with intensive brown color with blue shade, silvering of 50% up to 70% and quite good fur quality traits. Since 1995, the pastel fox is under national protection in order to store and maintain existing state of animals within the protection of genetic resources of domestic breed populations.

Conclusion

On a base of results achieved, the following conclusions were drawn:

1. Positive values of genetic trends for conformation traits and number of reared animals testify to proper direction of breeding. However, their low values point out to low efficiency of selection. It can be a result of large number of traits considered during selection.
2. For all investigated traits, with exception of litter size at birth, an increasing tendency was found during the years under investigation which demonstrates , the breeding work was conducted properly in this flock.

References

Brzozowski M.: 1995. Studia nad rozpłodem lisów w Polsce. Rozprawa habililitacyjna. SGGW Warszawa.

Groneveld E., 1998. VCE 4.2.5. User's Guide and Reference version 1.1.

Jeżewska G.: 1987 - Fenotypowa i genetyczna charakterystyka odmian barwnych lisa pospolitego (Vulpes vulpes L.) hodowanego w Polsce. Rozprawa habilitacyjna. Rozprawy naukowe. Wydawnictwo AR Lublin 105, 1-50.

Maciejowski J., 1983: Stan i perspektywy hodowli lisa pastelowego w Polsce. Zesz. Probl. Post. Nauk Roln. 302, 91-97.

Maciejowski J., Jeżewska G.: 1987. Wyniki pracy hodowlanej nad lisem pastelowym w latach 1981-1984. Zesz. Probl. Post. Nauk Roln. 341, 97-109.

Maciejowski J., Kasperek R., 1979: Wstępne wyniki pracy hodowlanej nad pastelową odmianą lisa pospolitego. Hod. Dr. Inw. 1979, nr 4, 4-6.

Misztal I., 1997: BLUPF90 – a flexible mixed model program in fortran 90. http://nce.ads.uga.edu/~ignacy/f90.
Pisański W.: Nowa odmiana barwna pastelowo srebrzysta lisa pospolitego. Hod. Dr. Inw. 1976, nr 9, 11-12.
Wzorzec oceny pokroju lisów pospolitych. CSHZ 1997

Suportem by KBN, grant No. 6 P06D 010 21

IV – 13 RP

Genetic variability of chosen conformation traits in Chinchilla

Grażyna Jeżewska, Iwona Rozempolska-Rucińska, Grzegorz Zięba
Faculty of Biology and Animal Breeding, Agricultural University of Lublin,
Akademicka 13, 20-950 Lublin
e-mail: furan@ursus.ar.lublin.pl

Summary
The purpose of the studies was to evaluate genetic determination in chosen performance traits in chinchilla. Nine generations of standard chinchilla population was taken into consideration. Conformation evaluation carried on 1565 animals (59 males and 1506 females). Body size, colour type, fur colour purity, fur quality and belly-belt were taken into account.
The components of the co-variance of conformation traits were evaluated by REML method, basing on the multiple-traits animal model by the DMU. Genetic analysis of particular traits were performed with respect to: random additive genetic effects of animal, random additive genetic effects of animal’s mother, permanent effects of year and month of whelping, sex and regression on the number of weaned offspring.
Heritability coefficients ranged from 0.071 to 0.389. The highest value concerned colour type (0.389) as well as fur colour purity (0.363). The mother’s additive effect on the level of examined conformation traits oscillated from 0.054 to 0.672.

Introduction
The profitability of farm breeding of fur animals, including chinchilla, with the production and sale of skins is connected. Both the number and the quality of the obtained product condition reaching the maximum profit. Constant improvement of the animals is possible only owing to adequately conducted breeding, the aim of which ought to be transforming the existing variability into the possibly highest raising progress. The basis of the effective breeding program should be a reliable control of the usability of the animals as well as the evaluation of their breeding value [Łukaszewicz & Krencik, 1992]. It requires learned the variability of the features improved by a breeder. The variability described by variance components based on which genetic and environmental parameters are calculated. The absence of clearly visible genetic variability may largely impede arriving at breeding progress. The value of the received evaluation is therefore the prognostic of the efficiency of the performed selection.
The objective of the conducted studies was the evaluation of genetic variability of the most important traits of body conformation of chinchilla standard variety.

Material and Methods
The material for the studies came from the documentation of a pedigree farm of chinchilla. The studies covered a nine-generation population of the animals, standard variety. In the research years, were the 1.565 animals (59 male and 1.506 female) evaluated. There were no yearly changes in the conditions the animals were maintained in on the farm. Chinchillas were fed according to the norm recommended for the phytophagous fur animals [9] and were subjected to appropriate prophylactic and veterinary regime.
Two different standards were used for evaluation of animal body conformation during the years of studies. Therefore, in the work all evaluation results were standardized according to the pattern recommended and utilized presently [1999]. The following characteristics were analyzed: body size, fur colour type, colour purity, fur quality and belly-belt (tab.1); the last feature characterizes belt colour, its breadth, the run of the colour line as well as the contrast of the colour.

Table.1. Factors considered in evaluation of genetic parameters of the population.

Feature	Type	Body size and build	Colour type	Colour purity	Hair quality	Belly-belt
Year of birth x Month of kittening	F	x	x	x	x	x
Sex	F	x	x	x	x	x
The number of the raised litter	C	x	x	x	x	x
Additive genetic effects of animal	A	x	x	x	x	x
Additive genetic effects of animal’s mother	M	x	x	x	x	x

The type of factor: F- fixed, C – regression, A and M – random with relationship matrix

The maximal note for body conformation was 30. Individuals receiving 0 (zero) points for any of the traits were disqualified as breeders and excluded from calculations. The evaluations were performed during the whole year on the animals aging 6 months at least. Among the analyzed features, only the chinchilla’s body size was analyzed objectively - by weighing the animals; the evaluations of the other traits were subjective.
The components of the co-variance of conformation traits were evaluated by REML method, basing on the multi-traits animal model, using the pack of DMU programs [Madsen & Jensen, 2000]. The factors considered in genetic analyses of particular features are presented in table 1. Owing to the fact, that the examined features have discrete variability, the probit conversions of the received parameters were used in calculations [Żuk, 1989].

Results and Discussion

The values of evaluated traits in studied chinchilla population (tab. 2) were not different from the results presented by other authors [Socha S, Olechno A, 2000].

Table 2. Mean value, heritability (h^2) and maternal effect (m^2) of studied conformation traits.

Trait	**Mean**	**S.D.**	**h^2**	**m^2**
Body size	3.0	0.3	0.1269	0.1310
Colour type	3.7	0.6	0.3887	0.1351
Colour purity	7.6	0.8	0.3625	0.1467
Fur quality	5.0	0.8	0.0709	0.0542
Belly-belt	2.9	0.3	0.2607	0.6722

The heritability coefficients of conformation traits evaluated in the study ranged from 0.071 to 0.389 (tab. 2). In the case of such features as body size and built as well as fur quality, the value of heritability coefficients showed only slight additive effect of the animal on the discussed characteristics. The absence of clearly visible genetic variability may greatly impede the breeding progress, especially when phenotype based selection would be performed

exclusively. In such a situation, the improvement in body size and build may be obtained by influencing environmental factors. However, considering the fact that both body built and fur quality significantly affect the prize of the obtained skins, special attention should be paid to the method of selecting animals in view of such features. Low level of heritability coefficients suggests that the selection of animals based on productive value was encumbered by a significant error. However, on the majority of home farms, this system is most often used. The selection of adequate individuals on the phenotype basis is the quickest and the simplest in farm conditions, however, the received ranging of the animals is conclusive only as for highly heritability factors [Falconer, 1989].

Additive value of an individual was a significant source of variability in the case of the remaining conformation traits in chinchilla. The highest values of the evaluated genetic parameters concerned the coloured type (0.389) as well as the fur colour purity (0.363). These features seem to be significantly susceptible to selection in the case of not only chinchilla but also other fur animals [Lagerkvist et al., 1994; Filistowicz & Żuk, 1995].

The additive maternal effect on the level of the chinchilla's conformation traits is presented in table 2. The additive value of the mother ranged from 0.054 to 0.672 and was a significant source of variability of examined traits. The performed analyses revealed that the maternal effect was important especially in the case of the breadth of the belly-belt (m^2 = 0.627) and body size (m^2= 0.131), exceeding genetic variability resulting from the additive value of a specimen. It shows then, that choosing animals for a selective herd, considering such features, mothers should be paid special attention to. Higher influence of a female, compared with a male, results not only from the indirect genetic and physiologic effects of genes determining the levels of particular traits, but also from the ability to transfer of the mitochondrial DNA genes (mitochondrial heredity) [Charon & Świtoński, 2000].

It seems the body size and build of the animals are determined largely by the maternal effect not only in the case of chinchilla. Similar results determining the influence of this factor on the variability of conformation traits were received in minks [Rozempolska – Rucińska, 2003]. The maternal effect in the case of mink body weight was 0.216, exceeding general genetic variability for this factor. In the studies conducted by Berg [1993a, 1993b], maternal effects ranged from 10 to 40% of the total variability of body weight and the size of mink skins.

Conclusions

Low value of heritability coefficients concerning the quality of fur as well as chinchilla body size and build shows that the selection of the animals based exclusively on their utility value may be subject to error, making it difficult to obtain breeding progress for these economically relevant features.

A significant source of variability of the studied traits appeared to be the additive maternal effect, which, in the case of such traits like belly-belt as well as body size and build of the animals, was considerably more important than genetic variability resulting from additive value of the individual.

References

Berg P.: 1993a. Variation within and between populations of mink. II. Skin and fur characteristics. Acta Agric. Scand., Sect. A, Anim. Sci. 43, 158-164.

Berg P.: 1993b. Variation within and between populations of mink. I. Weight and skin length. Acta Agric. Scand., Sect. A, Anim. Sci. 43, 151-157.

Charon K.M., Świtoński M.: 2000. Genetyka zwierząt. Wydawnictwo Naukowe PWN, Warszawa.

Falconer D.S., 1989. Introduction to quantitative genetics. Longman Scientific & Technical.

Filistowicz A., Żuk B.: 1995. Zastosowanie programów hodowlanych w doskonaleniu zwierząt futerkowych w Polsce. Zeszyty Naukowe Przeglądu Hodowlanego. 21, 55-67.

Lagerkvist G., Johansson K., Lundeheim N.: 1994. Selection for Litter Size, Body Weight, and Pelt Quality in Mink (Mustela vison}: Correlated Responses. Journal of Animal Science. 72, 1126-1137.

Łukaszewicz M., Krencik D.: 1992. Ogólne założenia metod wyceny wartości hodowlanej bydła mlecznego. Przegląd Hodowlany. 12, 22-25.

Madsen P., Jensen J., 2000. A user's guide to DMU - a package for analyzing multivariate mixed models. Version 6, release 4. Danish Institute of Agricultural Sciences.

Normy żywienia mięsożernych i roślinożernych zwierząt futerkowych. 1994. PAN, Instytut

Fizjologii i 1992. 7.Żywienia Zwierząt im. J. Kielanowskiego.

Rozempolska-Rucińska I., 2003. Genetic factors of mink utility and functional traits. In press.

Socha S., Olechno A., 2000. Analysis of changeability of features in chinchillas (*chinchilla velligera m.*). Electronic Journal of Polish Agricultural Universities, Animal Husbandry, 3, 2. http://www.ejpau.media.pl/series/volume3/issue2/animal/art-04.html

Wzorzec oceny pokroju szynszyli. Centralna Stacja Hodowli Zwierząt, 1999.

Żuk B. 1989. Biometria stosowana. PWN Warszawa.

IV – 14 RP

Genetic and phenotypic parameters of animal size and fur traits in common silver fox (*Vulpes vulpes* L.)

S. Socha, D. Kołodziejczyk, A. Gontarz
Department of Breeding Methods and Fur Animals Breeding, University of Podlasie, 08-110 Siedlce, ul. B. Prusa 12. Poland, e-mail: socha@ap.siedlce.pl

Abstract.
The aim of the work was to evaluate the parameters (heritability and genetic, phenotypic and environmental correlations) of common silver fox.. Coefficients were calculated from dam and sire variance components. Heritability coefficient was the highest for colour type: 0.900 and for other traits was as follows: animal size 0.065, purity of silver colour and colour purity 0.296, fur quality 0.547 and total number of scores 0.443. The genetic correlations ranged from -0.550 (between animal size and fur quality) to 0.900 (between animal size and total score). The phenotypic correlations had lower scope and ranged from -0.160 (between animal size and fur quality) to 0.470 (between animal size and total score). The environmental correlations were on the similar level. Obtained values prove that foxes of larger dimensions were characterised by lower quality of fur.

Introduction

The most important economic traits in carnivorous fur bearing animals are body size and fur quality. They influence decisively the effectiveness of breeding of animals. Genetic and phenotypic parameters of fur quality traits were researched by various authors (Maciejowski & Jeżewska, 1981; Kenttämies, 1988; Socha, 1994 and 1996; Filistowicz et al., 1999; Socha et al., 2000). However it should be pointed out that genetic and phenotypic parameters in populations change constantly due to the changes of genetic variability, which are caused by the selection and import of animals. Moreover, the parameters vary among different herds. In view of the mentioned aspects the present work estimates both heritability and genetic, phenotypic and environmental correlations between animal size and fur quality traits in common Silver Fox (*Vulpes vulpes* L.).

Material and methods

The data was obtained from the breeding farm of common silver fox (*Vulpes vulpes* L.) situated in Middle Poland. The data included the evaluations of body dimensions in the autumn (official evaluation period) within 2 years (2000-2001).

The traits analysed were as following: a. body size and conformation, b. colour type, c. purity of silver, colour purity and purity of silver (defined as colour purity), d. fur quality (hair length and fur density) and total score for all the evaluated traits. The animals were estimated according to the new rules (Wzorzec - Norm, 1997), on a point scale. The maximum score that could be obtained is 20 while the minimum 0 (zero) for a trait disqualifies an animal from further breeding. About 800 animals were recorded, 90% obtained positive results. Genetic parameters (heritability and genetic, phenotypic and environmental correlations) were estimated from sire and dam variance components. The mixed model was applied:

$$Y_{ijklm} = \mu + a_i + b_{ij} + r_k + p_l + e_{ijklm},$$

where: μ = overall mean, a_i = random effect of sire, random effect of dam, r_k = fixed effect of birth year, p_l = fixed effect of animal sex, e_{ijklm}= random error.

Results and discussion

The obtained parameters of heritability of traits are presented in Table 1. Heritability coefficient was the lowest for animal size: 0.065, which was lower from the results obtained by Filistowicz et al. (1999) and Socha et al. (2000). The low value proves that animal size is mostly influenced by environmental conditions, such as feeding and only a little by genetic base.

Heritability of other traits was as following: colour type 0.900; colour purity 0.296; fur quality 0.547 and total score 0.443. High heritability of fur colour was also obtained by Pingel et al. (1986) in mink: from 0.53 to 0.86, depending on animal sex and colour type. Borsting & Clousen (1986) estimated it: from 0.10 to 0.49, depending on animal sex and colour type. Heritability of fur quality (fur density and hair length) in this work was higher from the results obtained by both Kenttämies (1986 and 1988) and Borsting & Clausen (1986): from 0.10 to 0.38, depending on animal sex. The higher fur quality in

the present work proves an increase of genetic base on these traits, which should be recognised as very favourable.

Table 1. The heritability coefficients and the correlations coefficients in common silver fox (*Vulpes vulpes* L.)

Traits (number of trait)	Heritability h^2	Number of trait Genetic correlations Phenotypic correlations Environmental correlations			
		(2)	(3)	(4)	(5)
Animal dimension (1)	0.065	0.511	>1	-0.545	0.939
		0.017	-0.066	-0.160	0.472
		-0.848	-0.267	-0.088	0.433
Colour type (2)	0.900		0.081	-0.108	0.230
			0.057	-0.121	0.099
			0.114	-0.456	-0.515
Colour purity (3)	0.296			0.457	1.048
				0.005	0.438
				-0.335	0.094
Fur quality (4)	0.547				0.298
					0.429
					0.561
Total score (5)	0.443				

Table 1 presents correlations between analysed traits. On the whole, all the correlations between total score and the traits evaluated during the license were positive. Increase of value of every trait increased total score (Socha et al., 2000). All the correlations (genetic, phenotypic and environmental) were both positive and negative.

The lowest negative genetic correlation was found between animal size and fur quality (-0.545). Phenotypic correlation between these traits (-0.160) was lowest as well. Negative correlation between animal size and fur quality proves that the foxes of smaller dimensions were characterised by higher fur quality. Pingel et al. (1986) estimated phenotypic correlations between body mass and both fur colour and structure in mink as negative and low. Lohi & Hansen (1990) also obtained negative correlation between body mass and fur quality in mink.

It should be pointed out that genetic correlation between colour purity and fur quality was high and positive (0.457) while phenotypic correlation was low (0.005). The large differentiation of correlation coefficients (positive and negative) between traits in population of foxes makes the selection more difficult. Increase of value of one trait might cause decrease of values of other traits (Socha, 2000).

Conclusions

The estimated heritability was follows: for colour type: 0.900 (the highest), animal size 0.065, colour purity 0.296; fur quality 0.547 and total score 0.443. On the whole, the results were higher than those obtained by other authors. The values of correlations obtained in the present work prove that the foxes of bigger dimensions were characterised by lower quality of fur. From the other side, animal size significantly and considerably more than other traits influenced total score. It should be also pointed out that differentiation of correlations of foxes makes selection difficult, all the more so as in selection all the traits are important. Analysis of prices of skins on the skin auctions indicates that the trait that most significantly influences the price is animal size. Consequently this trait should be taken into special consideration during the selection. However, it is the trait that mostly depends on environmental conditions, such as feeding.

References

Borsting E., Clausen J. 1986: Evaluation of live animals in mink breeding. Rapporter, Nordiske Jordbrugsforskeres Forening 27, 2: 1-6.

Filistowicz A., Wierzbicki H., Zwolińska-Bartczak I. 1999: Genetic parameters of conformation and coat traits in fox (Vulpes vulpes) population. Journal of Applied Genetics 40, 3: 211- 217.

Kenttämies H. 1986: Inheritance of fur characters in the silver fox. Colloqium 110 on Fur Bearers, Kuopio, Finland, 9-11 sep 1986. Rapporter, Nordiske Jordbrug-sforskeres Forening 27, 4: 1-4.

Kenttämies H. 1988: Heritability of body size and fur quality in foxes (Vulpes vulpes). Biology, pathology and genetics of fur bearing animals. Proceedings of the IV International Congress in Fur Animal Production, Ontario, Canada, August 21-24, 1988: 548-556.

Lohi O., Hansen B. K., 1990: Heritability of body length and weight in mink and the effects of time of bith and litter size on growth. Nordisk Jordbrugsforskning 72, 1: 128.

Maciejowski J., Jeżewska G. 1981: Heritability of hair length in Arctic foxes. Zeszyty Problemowe Postępów Nauk Rolniczych 259: 11-22.

Pingel H., Schaumacher Z., Zunft G. 1986: Genetiche Analyse der Lenbendmasse und Fellgualitüt beim Netz. Arch. Tierz., Berlin 29, 1: 13-20.

Socha S. 1994: Genetic parameters of fur traits and body size in Arctic blue fox (*Alopex lagopu s* L.). Applied Science Reports of Polish Society of Animal Production 15: 19-28.

Socha S. 1996: The analysis of outcomes of breeding work on Polar blue fox (*Alopex lagopus* L.) at a farm. Agricultural and Pedagogic University Siedlce, Rozprawa naukowa 43.

Socha S., Jeżewska G., Gontarz A. 2000: The heritability and correlation coefficients of the selected traits in the population of common silver foxes (*Vulpes vulpes* L.). Scientifur, 24, 4, Genetics, Volume III-B: 123-126.

Wzorzec - Norm (1997): Norm of evaluation of conformation traits in fox (Wzorzec oceny pokroju lisów pospolitych) (official instruction). Central Animal Breeding Office. Warszawa.

IV – 15 RP

Genetic parameters of size and fur quality in a mink population (*Mustela vison* Sch.)

S. Socha

Department of Breeding Methods and Fur Animals' Breeding, University of Podlasie, ul. B. Prusa 12, 08-110 Siedlce, Poland, e-mail: socha@ap.siedlce.pl

Abstract

The aim of the work was to estimate the heritability and correlations between body size and fur quality, estimated in a breeding-farm of mink. Genetic parameters of the traits were obtained by the REML method with a multitrait animal model. Since the fur traits were evaluated on a discrete scale and distribution of scores differs from a normal distribution, probit transformations of the obtained heritability and phenotypic correlation estimates of traits were performed. Obtained heritability estimates: 0.515 for body size (based on point evaluation of animals) and 0.226 (based on body weight), 0.432 for colour purity, 0.387 for fur quality and 0.499 for the total score. The genetic correlations ranged from –0.125 (between body size and colour purity) to 0.802 (fur quality and total score). The phenotypic and the environmental correlations showed narrower ranges.

Introduction

Minks are the most widespread animals used in fur production. The most important economic traits are body size and fur traits. Heritability, as well as correlations between body size and fur quality of minks were reported by Børsting & Clausen (1986), Pingel et al. (1986), Einarsson (1988), Berg & Lohi (1991), Lagerkvist et al. (1994).

There is a large variation in heritability and correlation estimates among different mink populations. This variability is most probably caused by natural and artificial selection. The grading standard of minks was changed in Poland in 1997. The changes concerned the scale of scores (from 30 to 20 scores) as well as number of evaluated traits and the requirements concerning particular traits. The present paper reports on work done to estimate heritability and correlation between body size and fur quality in a mink farm.

Material and methods

Parameters (heritability and correlations) were estimated on the basis of the data obtained from a mink farm (Standard colour type) in central Poland, in the period of 2 years. The evaluation of body dimensions was done in the autumn (official evaluation period). The traits analysed were: a. body size and conformation, b. colour purity, c. fur quality (hair length and hair density) and total score for all the evaluated traits.

The evaluation of traits was on a point scale. Body size was recorded in grams, even though the norm does not require it (Wzorzec – Norm, 1997). However, it was done for comparison in this work. The maximum score which could be obtained was 20, and the minimum was 0 (zero) for the traits that disqualified animals from further breeding. During the experiment 3877 animals were recorded. Positive results were obtained for 3440 minks (1597 in the first year and 1847 in the second), 2382 females and 1058 males.

The parameters of the traits (heritability and correlations, phenotypic and genetic) were estimated by the REML method with a multitrait animal model. The model took into account the fixed effects: year of evaluation, sex, litter size and random effects: animal and error. The estimates of the variance and covariance components were obtained using SAS/STAT (1998) procedure**.**

Results and discussion

Means and standard deviations of the analysed traits are presented in Table 1. The lowest standard deviation of the scored traits was observed in colour purity (0.71) and the highest in total evaluation (1.19). Heritability, as well as genetic, phenotypic and environmental correlations were estimated (Tables 1 and 2). The heritability found for body size was 0.515, based on the point evaluation or 0.226, when estimated on the basis of animal weights. Heritability estimates of body weight presented by other authors were the following: from 0.22 to 0.78, adjusted for gender and colour type (Pingel et al. 1986); 0.54, while body length was 0.72 (Lohi et al., 1990); between 0.20 and 0.44, depending on the method (Jeżewska & Maciejowski, 1981);

between 0.05 and 0.54, depending on the animal age (Lohi & Hansen, 1989). The high values suggest that selection for increasing body weight of minks should be very effective under suitable conditions, especially suitable feeding.

Table 1. Means, standard deviations and heritability coefficients of conformation traits in mink

Traits	Material		Results	
	Mean	Standard deviation	Heritability h^2	Standard errors of heritability – SE_h^2
Body weight; g	1523	476.8	0.226	0.041
Body size and conformation; scores evaluation	5.49	0.666	0.515	0.061
Colour purity	4.44	0.702	0.432	0.050
Fur quality	5.09	0.730	0.387	0.049
Total score	18.02	1.191	0.499	0.053

Estimates of heritability of fur colour purity, fur quality and total score were 0.432, 0.387 and 0.499, respectively. Heritability of fur traits obtained by other authors varied and was the following: between 0.53 and 0.86 for the hue, depending on gender and colour type (Pingel et al., 1986); between 0.10 and 0.49 for fur colour, between 0.10 and 0.38 for fur quality, between 0.32 and 0.79 for body size (estimates related to the gender and colour type, Børsting & Clausen, 1986); between 0.18 and 0.53 for body weight at pelting, between 0.52 and 0.72 for fur colour, between 0.24 and 0.93 for hair length, between 0.33 and 0.49 for hair density and between 0.15 and 0.31 for general fur quality among several mink lines (parameters from the sire variance components, Einarsson, 1988).

In the Table 2 the estimated genetic, phenotypic and environmental correlations between the investigated traits are presented. It should be generally ascertained that all correlation coefficients between total score and the traits particular evaluated during the official evaluation were positive and statistically significant. The increase of the values of each trait influenced the boost of the total score. It was found that the total score (r_G = 0.802) than between total score and colour purity (r_G = 0.584).

The majority of the genetic correlations were positive, negative estimates were found, for instance, between colour purity of fur and body size (-0.030 for body weight and –0.125 of point evaluation for body size). Po sitive correlations ranged from 0.268 (between colour purity and fur quality) to 0.802 (between fur quality and total score).

Table 2. Estimated correlation coefficients between the traits studied in mink

Traits	Correlation estimates			
	Body size and conformation	Colour purity	Fur quality	Total score
Body weight; g	0.693 [a] ** (0.055 [d]) 0.815 [b] ** 0.640 [c] **	-0.030 [a] (0.110 [d]) -0.046 [b] -0.054 [c]	0.371 [a] ** (0.100 [d]) 0.091 [b] -0.019 [c]	0.534 [a] ** (0.083 [d]) 0.408 [b] ** 0.337 [c] **
Body size and conformation (scores evaluation)		-0.125 [a] ** (0.095 [d]) -0.137 [b] ** -0.081 [c]	0.309 [a] ** (0.096 [d]) 0.055 [b] -0.083 [c]	0.589 [a] ** (0.057 [d]) 0.693 [b] ** 0.495 [c] **
Colour purity			0.268 [a] ** (0.093 [d]) 0.011 [b] -0.105 [c] **	0.584 [a] ** (0.057 [d]) 0.669 [b] ** 0.530 [c] **
Fur quality				0.802 [a] ** (0.039 [d]) 0.733 [b] ** 0.542 [c] **

a) genetic correlation, b) phenotypic correlation, c) environmental correlation, d) standard errors of genetic correlation estimates

*** - correlation highly significant $\alpha \leq 0.01$*

Lagerkvist et al. (1994) estimated genetic and phenotypic correlations between certain traits of mink fur. The correlations between colour and density were low and negative (from -0.10 to –0.05). The correlations between colour and quality were also low but positive (from 0.06 to 0.18). Pingel et al. (1986) estimated negative and low phenotypic correlations between body mass and: fur colour and fur structure. Lohi & Hansen (1990) have found the negative correlations between body weight and fur quality of minks also.

The parameters of conformation traits estimated by REML method indicate that the traits are characterised by additive genetic variability. The achievement of breeding progress might, however, be difficult due to the various factors: the correlation coefficients differ, the right choice of animals for breeding will always be difficult, and the results of the selection will be hard to predict. Large differences between correlation coefficients (from negative to positive) between the conformation traits of minks is a serious problem in their selection. The increase in values of certain traits might cause decrease of others. A selection index might overcome some of the problems.

Conclusions

1. The highest values of heritability were obtained for body size (0.515 of point evaluation), other traits ranged from 0.226 (body weight) to 0.499 (total score). The estimated parameters (except body size) had higher values than those presented by other authors.
2. Negative genetic correlations between body size and other traits (fur purity) were obtained. The correlations between particular traits and total score were high and positive. Thus, selection on total evaluation should be made in order to achieve breeding progress in these traits. The differences between the correlation coefficients might be a serious problem i.e. in the selection of minks’ where the detailed estimation of breeding value of animals is essential.

References

Berg P., Lohi O. 1991: Hair length and skin thickness in mink. Genetic and environmental effects. Vara Palsdjur. 62 (5) 139.

Børsting E., Clausen J. 1986: Evaluation of live animals in mink breeding. Rapporter, Nordiske Jordbrugsforskeres Forening 27 (2), 1-6.

Einarsson E. J. 1988: Selection for litter size in mink-experiment and breeding program. Proceedings of the IV International Congress in Fur Animal Production, Ontario (Canada), 575- 583.

Jeżewska G., Maciejowski J. 1981: The heritability of live weight in standard minks, estimated according to different statistical methods (in Polish). Prace Mat. Zoot. 27, 7-15.

Lagerkvist G., Johansson K., Lundheim N. 1994: Selection for litter size, body weight and pelt quality in mink (*Mustela vison*): correlated responses. J. Anim. Sci. 72, 1126- 1137.

Lohi O., Hansen B. K. 1989: Heritability of body length and weight in mink and the influence of litter size and age on size development. Scientifur 13, 329-330.

Lohi O., Hansen B. K. 1990: Heritability of body length and weight in mink and the effects of time of birth and litter size on growth. Nordisk Jordbrugsforskning 72, 1, 128.

Lohi O., Hansen B. K., Krogh- Hansen B. 1990: Heritability of body length and weight in mink. Deut. Pelztierzüchter 64, 4-5.

Pingel H., Schaumacher Z., Zunft G. 1986: Genetische Analyse der Lenbendmasse und Fellqualität beim Nerz. Arch. Tierzucht. 29, 13- 20.

SAS/STAT 1998: User’s Guide. 6.03 Edition., SAS inst. Inc., Cary, NC, USA.

Wzorzec - Norm, 1997: Norm of evaluation of conformation traits in mink Wzorzec oceny pokroju norek (official instruction). Central Animal Breeding Office. Warszawa.

IV – 16 RP

Comparison of reproduction management intensity of three genetic lines of female chinchillas (*Chinchilla lanigera* M.)

Seremak B[1]., Sulik M.[2]
[1]Agricultural University of Szczecin, Department of Animal Reproduction,
[2 1]Agricultural University of Szczecin, Department of Fur-Animal Breeding

Abstract
The observations took place on a chinchilla farm located in West Pomerania, Poland, during 1991-2002 and included 359 females assigned to three basic groups that represented separate genetic lines: imports from Sweden (141 females), own-bred (98 females), and imports from Denmark (120 females). The females qualified for the studies had produced at least four litters of offspring during their reproductive life.
The main goal of these investigations was to determine an effect of birth interval on selected reproduction parameters of female chinchillas.
It has been found from the studies that the mean birth interval was 221 days, which demonstrates that the females had been extensively managed in terms of reproduction. birth interval that lasts 8-9 months positively influences litter size and is especially recommended for females that nurse large-size litters. In the Swedish line, a drop in services was observed in the fourth oestrus after delivery, as compared with the remaining genetic lines. The own-bred females achieved the worst nursing success for the young born from services during the post-lactation oestrus, while the Swedish females – for those born from services during the fifth oestrus post-partum.

Introduction
Properly managed reproduction within the herd is the key factors of profitability of each farm. Producing numerous and healthy brood that achieves desired traits is the objective of every farmer, as in fur-bearing animals farming it is immediately associated with the number of produced pelts (Jeżewska et al. 1998, Socha and Szumska 2002).
Chinchillas belong to the animals of relatively low fertility and, which they descend from their wild ancestors, of distinct seasonality of reproduction (Nordholm 1992, Kuroiwa and Imamichi 1977, Seremak and Sulik 2002). Intensive sexual activity, which can be observed on farm, occurs depending on climatic conditions; in Poland this period falls between November and May (Gromadzka-Ostrowska 1998, Jarosz 1993). The periods of intense libido and successful services reach their peak in January and February, which results from longer days. According to Barabasz et al. (2000), seasonality in chinchilla farming begins to diminish, which may demonstrate better and better adaptation of the species to farming environment. Reproduction performance parameters achieved on Polish farms (1.7-2.4 born and 1.5-2.1 weaned young per female per year) should not be considered as fully satisfactory (Sulik and Barabasz 1995), especially as chinchilla reproductive potential is much higher (4-6 follicles maturate in the oestrous cycle).
Improvement of chinchilla herd in Poland is to a large extent based on imported animals, which are supposed to positively influence coat quality of pelts. The import makes an opportunity, however, to also improve reproduction performance. This study is aimed at an analysis and comparison of reproductive careers of females belonging to three genetic lines imported in order to improve performance of domestic lines.

Material and Methods
The data was collected over 11 years, i.e. from 1991 till 2002 and included observations of 359 females assigned into the following genetic groups by their origin:

- own-bred females, 98 animals
- imported from Sweden, 141 females
- imported from Denmark, 120 females

The females qualified for the analysis had produced at least four litters of offspring during their reproductive life.
The females were managed in polygamous sets system with male-to-females ratio like 1:4. Females in each set inhabited their own cages connected with a passage, so the male could access each female of the set. From 48 hours after birth, the passageway of the female' cage was closed. The animals were fed on balanced pellets with water and hay ad libitum. Additionally, each cage was equipped with a dust bath. The animals were housed without bedding, their cages being arranged in a four-storey system.
The analysis was based on farm documentation recordings. The females were within each genetic

group divided into subgroups according to birth interval length:

1. females serviced during the post-partum oestrus (birth interval shorter than 4 months)
2. females serviced during the post-lactation oestrus (birth interval between 5 and 6 months)
3. females with birth interval between 7 and 8 months
4. females with birth interval between 8 and 9 months
5. females with birth interval between 9 and 10 months
6. females with birth interval longer than 10 months

Another criterion of division was the number of litters per female achieved over her reproduction life.

The collected data was subjected to statistical analysis using a spreadsheet and Statistica PL software package.

Results and Discussion

Polish chinchilla farms have been nourished with imported quality animals in order to improve performance parameters, especially in terms of fur quality. Reproduction parameters, however, cannot be ignored in the process of stock improvement, since they significantly affect the profitability of the farm. According to many authors (Felska and Brzozowski 2001, Jarosz and Rewska 1996, Sulik et al. 2001), low fertility of chinchillas is a characteristic of the species reproduction and reaches 2 young per litter in most cases. On the analysed farm, fertility levels were achieved similar to those reported in the literature (2.12 per Polish, 2.01 per Danish, and 2.17 per Swedish female). These results do not show statistically significant differences in relation to the country of origin.

An average number of litters produced per female per year represents a factor that can bring high level of reproduction performance. Considering the gestation length, as well as that females are able to conceive during the post-partum oestrus, it is theoretically possible to obtain 3 litters per year from a single female. The number if litters depends on the interval length between births. Mating during the post-partum oestrus allows obtaining the highest index of female reproduction intensity. In the studied herd (Table 1), from 29.11% matings (own-bred females) to 40.78% (Swedish import) were done during post-partum oestrus, which may demonstrate an intensive utilisation of females; it should be noted that domestic females definitely differ from imported females in this area.

Analysis of litter size in relation to birth interval reveals a high level of variability of females' responses to the examined factor. Danish-bred females gave larger litters from post-lactation matings, while domestic and Swedish females produced larger sizes after an about 8-month break. From the economic point of view it seems better when a female have shorter breaks between parturitions. Although a female usually delivers fewer offspring per such a litter, the overall number of born young over a year is higher.

Table 2 presents intensity of female reproduction in relation to the number of litters attained. Gestation intervals in the females decrease with their age, irrespective of their origin. The mean birth interval was from 206 days in won-bred females to 238 days in Danish females. As it has been shown by Neir et al. (1989), who studied the chinchilla herd in Chile, an average gestation interval there was 212 days, which is similar to those achieved on the studied farm. This level allows obtaining 1.5 litters from a female per year on average. Considering the weaning success at the level of 1.88 to 1.97 (Table 1), 2.82 to 2.96 offspring, depending on the origin, were achieved from a female per year on the studied farm. This result is relatively high, as a number of authors state that obtaining 2-2.5 young chinchillas per female per year is considered a satisfactory achievement (Sulik 2001, Sulik and Barabasz 1995, Sulik et al. 2001). The above mentioned data demonstrates that gestation interval, which depends on the resting period length, represents an unusually important index of female reproduction management intensity.

In all the genetic lines, birth intervals were found to become shorter after each consecutive litter, with the eight litter as the limit of this effect (Table 2). Similar is the size of litters, which grows slightly until the eight or ninth litter to drop again by 0.15 to 0.54 young on average in the subsequent litter. However, as long as litter size exceeds two, a result can be considered as very good. The highest pre-weaning mortality rate was found among the Danish females (0.2 young per litter).

Conclusions

1. Birth interval lasting about 8 months positively influences litter size, however on the whole it results in reduced number of offspring obtained per year.
2. The birth interval of 208 to 238 days recorded on the studied farm shows that the females were extensively utilised in terms of reproduction.

3. On the studied farm, a female was on average utilised for 1270 days (3.5 years). This period looks to have been too short, since reproductive intensity was found to increase with the length of their reproductive life.

References

Barabasz B., Fortuńska D., Bieniek J., 2000, Ocena intensywności użytkowania rozpłodowego szynszyli. [Evaluation of chinchilla reproduction management intensity] Zesz. Nauk. AR Kraków 369 (35), s. 121-133 [In Polish].

Felska L., Brzozowski M., 2001, Porównanie wyników rozrodu trzech grup genetycznych szynszyli. [Comparison of reproduction performance of three genetic groups of chinchillas] Zesz. Nauk. Przegl. Hod. 58, s.31 [In Polish].

Gromadzka-Ostrowska J., 1998, Studia nad fizjologią szynszyli ze szczególnym uwzględnieniem rozrodu i odporności. [Studies on chinchilla physiology with particular consideration of reproduction and immunity] Zesz. Nauk. AR w Krakowie, Kraków [In Polish].

Jarosz S., 1973, The sexual cycle in chinchilla. Zoologia Poloniae, 23, s. 119-128

Jarosz S., Rżewska E., 1996, Szynszyle. Chów i hodowla. [Chinchilla; Farming and Breeding] PWRiL, Warszawa [In Polish].

Jeżewska G., Tarkowski J., Ślaska B., Jakubczak A., 1998, Wyniki rozrodu szynszyli różnych odmian barwnych. [Reproduction performance of various colour types chinchillas] Zesz. Nauk. AR w Lublinie [In Polish].

Kuroiwa J., Imamichi T., 1977, Growth and reproduction of the chinchilla age at vaginal opening, oestrus cycle, gestation period, litter size, sex ratio and diseases frequently encountered. Jikken Dobutsu, 26 (3), s. 213-222

Neira R., Garcia X., Schen R., 1989, Reproduction and growth in continent chinchillas (Chinchilla lanigera). Advances en Production Animal, Chile, 14(1-2), s. 109-119

Nordholm J., 1992, Studies on the period from first mating to parturition in young chinchilla females, Vara Pelsdjur 63 (3), s. 91-92

Seremak B., Sulik M., 2002, Sezonowa aktywność rozrodcza samic szynszyli na wybranych fermach. [Seasonal reproductive activity of chinchilla females on selected farms] Zesz. Nauk. Przegl. Hod. 64, s. 89-96 [In Polish].

Socha S., Szumska K., 2002, Analiza opłacalności chowu szynszyli w fermie reprodukcyjnej. [Analysis of profitability of chinchilla reproductive farming] Acta Scien. Polonorum 1 (1-2), s. 155-161 [In Polish].

Sulik M., Barabasz Helska., 1995, Porównanie systemów użytkowania rozpłodowego szynszyli na przykładzie wybranych ferm. [Comparison of reproductive management systems of chinchillas exemplified by selected farms] Zesz. Nauk. AR Helska Krakowie 297, Zoot. 30, Helska. 159-166 [In Polish].

Sulik M., Seremak B., Bielińska A., Mieleńczuk G., 2001, Intensywność użytkowania rozpłodowego samic szynszyli w wybranej fermie na Pomorzu Zachodnim. [Reproductive utilisation intensity of chinchilla females on a selected farm in West Pomerania] Zesz. Nauk. Przegl. Hod. 58, s. 73 [In Polish].

Table 1. Distribution of matings in relation to origin and birth interval

Mean birth interval	Number of litters						Litter size									Weaned young per litter								
	Polish		Swedish		Danish		Polish			Swedish			Danish			Polish			Swedish			Danish		
	Ind.	%	ind.	%	ind.	%	M	SD	V	M	SD	V	M	SD	V	M	SD	V	M	SD	V	M	SD	V
Until 5 months (post-partum oestrus)	122	29.11	219	40.78	202	37.33	2.19	0.97	44.3	2.03	0.84	41.4	2.13^{b}	0.82	38.5	1.97	0.94	47.7	1.86	0.85	45.7	1.92	0.84	43.6
5-6 months (post-lactation oestrus)	12	2.86	8	1.49	9	1.66	1.91	0.9	47.1	2	0.53	26.5	2.33	1	42.9	1.66	0.98	58.7	2	0.53	26.5	2.11	0.78	36.9
6-7 months	34	8.11	30	5.58	37	6.83	1.97	0.63	32	2	0.87	43.5	2.27	0.73	32.2	1.97	0.67	34	1.86	0.93	50	2	0.94	47
7-8 months	97	23.15	12	2.23	109	20.14	2.14	0.86	40.2	2.06	0.7	34	2.05	0.84	40.8	1.98	0.84	42.4	1.92	0.71	52.1	1.85	0.81	42.9
8-9 months	37	8.83	72	13.4	48	8.87	2.32	0.88	37.9	1.91^{a}	0.78	40.8	2.43^{ab}	0.79	32.1	2.08	0.72	34.6	1.79	0.71	39.7	2.14	0.74	34.4
9-10 months	33	7.87	41	7.63	33	6.09	2.18	0.88	36.7	2.14	0.76	35.5	2.03	0.98	42.6	2.03	0.8	39.4	1.97	0.68	34.5	2	0.93	46.5
10 and more	84	20.04	155	28.86	103	19.03	2.13	0.67	31.5	1.94	0.69	35.6	2.01	0.76	37.8	2.02	0.72	35.6	1.81	0.68	37.6	1.82	0.78	42.6
Total	419	100	537	100	541	100	2.12	0.82	38.7	2.01	0.73	36.3	2.17	0.85	39.2	1.95	0.87	44.6	1.88	0.72	38.3	1.97	0.83	42.13

Table 2. Intensity of reproductive management of female chinchillas in relation to origin

Origin	No. of litter	No. of females		Mean length of reproductive life (months)			Mean birth interval (days)	Mean litter size (indiv.)			Mean number of weaned young per litter (indiv.)		
				M	min	max	M	M	S	V	M	S	V
Polish	4	40	40.81	667	340	1217	222	2.19	0.8	36.5	2.02	0.81	40.1
	5	27	27.55	959	451	1856	239	1.99	0.75	37.7	1.85	0.71	38.4
	6	7	7.14	1277	731	2465	255	2.09	0.84	40.2	1.95	0.88	45.1
	7	16	16.32	1203	927	2035	200	2.17	0.93	42.9	2	0.88	44
	8	5	5.1	1198	996	1569	171	2.3	1.01	43.9	2.12	0.93	43.9
	9	2	2.04	1397	1241	1553	174	2.44	0.92	37.7	2.11	0.75	35.5
	10 and more	1	1.02	1623	1623	1623	180	1.9	0.73	38.4	1.7	0.67	39.4
	Total	98	100	1189	340	2465	206	2.15	0.85	39.5	1.96	0.8	40.8
Swedish	4	50	35.46	672	340	1456	224	1.96	0.77	39.3	1.8	0.76	42.2
	5	40	28.37	967	522	1835	241	2.04	0.78	38.2	1.9	0.78	41
	6	13	9.22	1198	769	1883	239	2.01	0.81	40.3	1.85	0.78	42.2
	7	19	13.47	1348	829	1868	224	2.03	0.76	36.4	1.86	0.84	50.6
	8	8	5.67	1489	1137	2491	212	2.09	0.79	37.8	1.95	0.74	37.9
	9	4	2.83	1653	1207	2265	206	1.94	0.58	29.9	1.86	0.72	38.7
	10 and more	7	4.96	1819	1322	2105	181	2.07	0.72	34.8	1.92	0.7	36.5
	Total	141	100	1307	340	2491	218	2.02	0.74	36.6	1.88	0.76	40.4
Danish	4	39	32.5	706	342	2404	239	2.05	0.8	38.8	1.78	0.79	43.6
	5	43	35.8	790	436	2331	197	2.19	0.81	37	1.92	0.85	44.3
	6	15	12.5	1146	626	2822	382	2	0.7	35	1.83	0.81	44.3
	7	9	7.5	1379	664	3189	229	2.25	0.76	33.8	2.03	0.71	34.9
	8	3	2.5	1405	956	1968	200	1.79	0.77	43	1.7	0.75	44.1
	9	4	3.3	1685	1370	1901	210	2.52	1.1	43.7	2.19	1.03	47
	10 and more	7	5.83	2117	1581	3100	211	2.01	0.8	39.8	1.96	0.81	41.3
	Total	120	100	1318	342	3189	238	2.12	0.82	38.7	1.92	0.82	42.7

IV – 17 RP

Morphological changes of spermatozoa in breeding raccoon dogs semen during cryopreservation.

Niedbala P., Szeleszczuk O.
Agricultural University of Cracow, Faculty of Animal Breeding and Biology, Al. Mickiewicza 24/28 30-150 Cracow, Poland.

Abstract
Process of dilution and semen freezing provides in a smaller or bigger degree damage of cell membrane of spermatozoa, thereby decreasing its fertilization ability. We provide investigations which aim was to evaluate the damage degree of raccoon dog spermatozoa during freezing process, after administration of different extenders. Investigations were carried out on semen collected manually from 16 raccoon dog males. After evaluation, semen was diluted with EDTA extender with 4, 6 and 10 % glycerol addition. Morphology of spermatozoa was evaluated on thin smears on a slide stained with 5% eosin and 10% nigrosin (1:4 v/v). Spermatozoa normal and not damaged in fresh semen were 63%. Among those with secondary changes a majority of 34.3% were spermatozoa witch proximal droplets. In frozen-thawed semen smears, the observed highly significant decrease of intact spermatozoa was dependent on glycerol addition. Up to 52-54% highly significant increase of spermatozoa with coiled tail and “hair pin” was observed.

Research was supported by the grant from the State Committee for Scientific Research Nr 2 P606Z046 26

Introduction
The process of semen dilution and freezing provides in a smaller or bigger degree to damaging of cellular membrane of spermatozoa, thus decrease their ability to fertility [Aquirre et al. 1987]. Considerable step during semen freezing is not only testing semen vitality after freezing, but also to estimate the degree of spermatozoa changes after cryopreservation [Bittmar et Kosiniak 1992]. During cryopreservation some structural changes of spermatozoa can be observed. In bulls’ semen, vitality test is based on semen mobility [Olar et al 989]. In the boar semen, the acrosome is more susceptible for damage, but not mitochondria in interstitial lamella. After boar semen freezing, spermatozoa show mobility but without biological value due to acrosome damage [Strzezek 1995].] We undertook investigations which aim was evaluation of raccoon dog spermatozoa damage during freezing process with different addition of cryoprotector.

Material and Methods
Investigations were carried out on semen collected after digital manipulation from 16 breeding males of raccoon dogs. Semen was collected and transported in containers at temperature 37ºC. After micro and macroscopic semen evaluation, selected ejaculates were diluted using EDTA extenders of composition given in Table 1. Semen was diluted gradually in glass tubes to obtain final concentration of spermatozoa $150x10^6$ per ml. The diluted semen was cooled and equlibrated at 5^0C. Semen was freezed using MINITUB system using marked 0.5 ml PVC straws which were filled up using syringes cooled down to 4 ºC. To freeze the semen, a polystyrene box with 4.5 cm thick walls and internal dimensions of container 17.5 x 17.5 x 19 cm was used. Straw hanger was placed in the container to keep them in N_2 vapor 4 cm above the liquid nitrogen; after 15 minutes, frozen semen was sunk and stored in GT-14 container (L’air Liquide, France). Frozen samples were thawed in a water bath ST-1 (OSTA Electrinics, Piastów, Poland) at 37ºC during 30 seconds immediately before examination. Spermatozoa morphology was evaluated on a stained 5% eosin and 10% nigrosin (1:4 v/v) slides under immerse magnification using light microscope Bioval. On each slide, at least 200 spermatozoa were evaluated. Number of intact spermatozoa and morphologically abnormal spermatozoa with secondary changes, with cytoplasmatic droplet, with twisted tail, damaged, with a changed cap (acrosome) and aggregated, was evaluated [Blom, 1981]. Statistical analyses of the results were conducted using SAS version 6.03.1987. Distribution of variables was tested. The significance of difference between extenders for variables with regular distribution was tested using analysis of variance and the method of the least squares. Significance of differences between groups for variables with non-regular distribution was tested using non-parametric test of Kolmogrov-Smirnov. Also, correlation between variables using

Spearman method for non-regular distribution variables and Person’s method for variables with regular distribution was calculated.

Results and Discussion

The most important problem during semen freezing is keeping progressive motility of spermatozoa as well as enzymatic proteins of acrosome and interstitial lamella. Protein denaturation caused by low temperatures corresponds with decreasing activity of a few cellular enzymes responsible for fertilization ability of spermatozoa [Strzeżek 1998]. The process of dilution and conservation of raccoon dog semen significantly influenced the spermatozoa morphology. After semen thawing, regardless of applied diluents, a highly significant lower number of normal spermatozoa in all samples were observed. The highest decline from 62.75% to 51.76% was observed when diluent’s RII with 6% glycerol addition was tested. The presence of normal non-damaged spermatozoa in diluents with 4% (RI) and 10% (RIII) of glycerol addition was slightly higher - 53.75% and 54.97% respectively (Table 1).

Table 1. Composition of EDTA extenders

Components	Extender R-I	Extender R-II	Extender R-III
EDTA (g)	0,37	0,37	0,37
Citric acid (g)	0,375	0,375	0,375
Acid sodium carbonate (g)	0,12	0,12	0,12
Glucose (g)	6,0	6,0	6,0
Distilled water (ml)	Ad 100	Ad 100	Ad 100
Glycerol (ml)	4,0	6,0	10,0
Egg yolk (%)	20	20	20
Osmolarity mOsm	445	446	460

Strzeżek et al. [1984] concluded that during cryoconservation, morphological changes can be observed in the spermatozoa structure causing release of enzymatic proteins. This process is responsible for considerable lowering of semen biological value. In present studies, we investigated glycerol addition for freezing and thawing of raccoon dog semen. Glycerol is generally considered to be the best cryoprotector. Optimal level of glycerol in diluent’s is a compromise between its protective and toxic functions [Olar et al. 1989]. The level of toxic influence of glycerol on spermatozoa depends on its concentration and the time of contact with cell, and refers to changes in plasmolemma transmission as a result of denaturation influence of this compound on glycoprotein complexes of cellular membrane. On smears, a highly significant lowering of spermatozoa with a protoplasmatic droplet was observed. The highest decline from 7.94 % in the fresh semen to 0.56 % post-thaw was observed in R-III; the lowest decline in R-II (Table 2).

During freezing and thawing, a highly significant increase of spermatozoa with a coiled tail was observed (from 14.13 % in fresh semen to 23 % after thawing). Differences between applied diluents were not significant (Table 3). Number of spermatozoa with helically coiled tail increased from 5.75 % in fresh spermatozoa to about 16 % after thawing. The least damages were observed in R-I. In the complex cryobiochemical investigations of bull and boar semen it was stated that in the first minutes of spermatozoa contact with diluent’s containing glycerol, there can be observed a glycerol activation effect or „dilution effect”, which appeared as an intensive degradation of enzymatic proteins related to plasmolemma or presented in acrosome and tail of spermatozoa (Strzeżek, 1987). After thawing, a decreased number of damaged spermatozoa on smears can be observed; the highest (mean by 39 %) was present in samples of semen diluted with R-III and R-I.

Table 2. Morphology of spermatology in raccoon dogs semen during freezing/thawing

Semen	Items	W1	W2	W3	W4	W5	W6	W7
Fresh	Mean	62,75	7,94	14,13	5,75	4,50	0,44	1,44
	SE	1,66	0,90	1,37	0,62	0,53	0,20	0,29
	SD	6,65	3,61	5,49	2,49	2,13	0,81	1,15
	V%	10,60	45,42	38,85	43,30	47,32	186,04	80,20
Diluted with Extender R-I	Mean	53,76	0,95	23,14	16,26	3,51	1,38	1,00
	SE	2,52	0,23	1,29	1,92	0,58	0,39	0,27
	SD	10,10	0,93	5,15	7,68	2,31	1,54	1,10
	V%	18,78	99,06	22,27	47,27	65,98	112,28	109,55
Diluted with Extender R-II	Mean	51,76	1,07	23,32	16,32	4,14	1,32	2,07
	SE	2,32	0,56	1,30	1,74	0,86	0,38	0,57
	SD	9,27	2,24	5,19	6,95	3,44	1,54	2,29
	V%	17,91	210,37	22,25	42,57	83,45	117,11	111,23
Diluted with Extender R-III	Mean	54,57	0,57	23,70	16,69	2,76	0,95	0,76
	SE	1,86	0,20	1,29	1,84	0,53	0,35	0,23
	SD	7,42	0,81	5,17	7,36	2,11	1,39	0,93
	V%	13,60	144,70	21,84	44,13	76,85	148,15	124,13

W1- Intact spermatozooa, W2- With a protoplasmatic drop W3 - Bent tail W4 - Coiled tail W5 – Disintegrated W6 - Acrosomal defect W7 – Agglutinations

Table 3. Test of extender effect on the sperm morhology in fresh and freezing/thawing semen

Semen	Spermatozoa	R-I	R-II	R-III
Extender R-II	Intact	0,6994		
	With a protoplasmatic drop	0,9412		
	Bent tail	0,9202		
	Coiled tail	0,9996		
	Disintegrated	0,9996		
	Acrosomal defect	0,9999		
	Agglutinations	0,6994		
Extender R-III	Intact	0,4154	0,0935	
	With a protoplasmatic drop	0,9412	0,9996	
	Bent tail	0,7637	0,8411	
	Coiled tail	0,9999	0,9996	
	Disintegrated	0,9996	0,6994	
	Acrosomal defect	0,6994	0,9412	
	Agglutinations	0,9996	0,6994	
Świeże	Intact	0,0039**	0,0010**	0,0020**
	With a protoplasmatic drop	0,0001**	0,0001**	0,0001**
	Bent tail	0,0001**	0,0001**	0,0001**
	Coiled tail	0,0010**	0,0002**	0,0002**
	Disintegrated	0,0366*	0,0935	0,0366*
	Acrosomal defect	0,4154	0,4154	0,6994
	Agglutinations	0,6994	0,6994	0,2106

** Statistically significant at $P < 0.05$*
*** Statistically significant at $P < 0.01$*

Conclusion

Based on our preliminary observations, it can be stated that raccoon dogs semen is unusually sensitive to thermal shock. Insufficient protection is responsible for spermatozoa structure damage, in particular of acrosome and tail.

References

Aquirre S.M., Capaul E., De Luca L. 1987. Morphological and biochemical evaluation of fresh and cold-shocked dog semen. Veterinaria Argentina, 4, 35, 433-439.

Bittmar A., Kosiniak K. 1992. The role of selected biochemical components of eguine seminal plasma in determining suitability for deep freezing. Polish Archive of Veterinary, 32, 1-2, 17-25.

Blom E. 1981. Ocena morfologiczna wad plemników buhaja. Zmiany patologiczne plemników w świetle nowych badań. Medycyna Weterynaryjna, 37, 239-242

Olar T.T., Bowen R.A., Pickett B.W. 1989. Influence of extender, cryopreservation and seminal processing procedures on post thawing motility of canine spermatozoa frozen in straws. Therioganelogy, 31, 2, 451-461.

Kosiniak K., Bittmar A. 1994. Prognosis of dog semen freezability: biochemical markers in seminal plasma. ARTA, 5, 129-131.

Strzeżek J. 1996. Molekularne aspekty konserwacji nasienia knura. W; Andrologia w rozrodzie zwierząt. IZ i PAN Kraków, 90-107.

Strzeżek J. 1995. Fizjologia i biochemia struktur plemnika ssaka. W: Łukaszyk A., Bilińska B., Kowiak J., Bielańska-Osuchowska Z. Ultrastruktura i funkcja komórki. PWN, Warszawa, 99-126.

Strzeżek J. 1987. Wybrane układy enzymatyczne nasienia zwierząt gospodarskich w aspekcie doskonalenia jego konserwacji i płodności samców. Zeszyty Problemowe Postępów Nauk Rolniczych, 340, 9-40.

Strzeżek J., Glogowski J., Magierska E., Luberda Z., Jabłonowska C., 1984. Some aspects of cryobiochemistry of boar semen10th International Congress of Animal Reproduction and A.I., Urbana, II, 244.

IV – 18 RP

Induction of estrus and ovulation in breeding chinchilla by GnRH analogues

Olga Szeleszczuk, Katarzyna Rysak, Beata Seremak,*
Agricultural University of Cracow, Faculty of Animal Breeding and Biology, Department of Anatomy, rzszeles@cyf-kr.edu.pl,
**Agricultural University of Szczecin, Department of Animal Reproduction, Poland*

Abstract
Compared to other multiparous rodents the chinchilla have relatively low fecundity. During the heat in ovaries of sexual mature chinchilla we can observe up to 16 ovary follicles, of which only 4 ovulated. The possibility to increase fertility and fecundity of breeding chinchilla by using hormonal specimens containing busereline – a synthetic analogue of hypothalamus hormone – GnRH was investigated. First and second stage of the experiment was conducted on 48 females, which were randomly divided into 3 groups: 2 experimental and 1 control. Each experimental female was inoculated intramuscularly with 0,2 ml of Receptal Intervet – Group I; Bioreline Biochef – Group II, what correspond to 0,85 µg of busereline. Control group – Group III was inoculated intramuscularly with 0,2 ml of *Aqua pro injection* (Polfa). In the first stage of experiment, the effect of busereline administrations was evaluated by structural changes observed in ovaries of 24 females. In a second stage the remaining 24 females were housed for 60 days together with males. Results from the first stage showed that both GnRH analogues caused ovulation, and the female's ovaries showed presence of corpus luteum. Confirmation of this fact was kittening of females from groups: II and I in the second stage. Application of *placebo* in the control group did not induce folliculogenesis or ovulation.

Research was supported by the State Committee for Scientific Research as a Solicited Project PBZ-KBN-084/P06/2002 from 2003 to 2005 year

Introduction

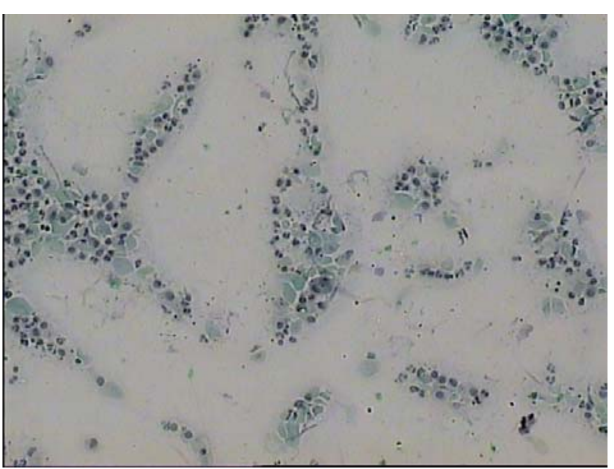
Chinchilla (*Chinchilla lanigera*) is a member of the rodent suborder *Hystricomorha* and bears one of the most valuable pelt in the world. The chinchilla, having great biological potential, show relatively low fertility and fecundity rates in comparison with other multiparous rodents [Jarosz & Rzewska, 1996]. During the sexual cycle up to 16 mature follicles which only 4 ovulate have been observe of in ovaries of mature chinchilla females, so theoretically a female can bear 4 kittens in each litter [Jarosz, 1969]. However, actual data are not in line with this theory. According to available information collected from chinchilla breeders, they have 2-4 kittens from one reproduction female per year [Jarosz, 1969]. Although chinchilla female can have two or even three litters a year from, however such a breeding system is not recommended [Wilk, 1989]. The difference between reproductive potential illustrated by the number of ovulating follicles and the low fecundity of chinchilla makes to introduction biotechnological methods into the practice. Such similar methods that have been successfully used for stimulation of estrus and ovulation in other farming animals [Bielanskii & Tischner, 1996; Kramer 1980]. The authors conducted experiment in order to induce estrus and ovulation in chinchilla females by a synthetic analogue of hypothalamus hormone GnRH.

Material and method

The experiment was conducted in two stages at the Experimental Farm Agricultural Academy of Cracow and at the RABA chinchilla farm in Myslenice near Cracow. In each stages, 24 adult and sexually mature females of standard variety were used. All of them were in good health condition and were fed with pellets applied according to their age and breeding phase. The first stage of experiment started in February. A group of 24 one-year old females were randomly divided into three groups at 8 females each: two experimental and one control. Each female from Group I was intramuscularly inoculated with 0,2 ml Receptal Intervet, which correspond to 0,84 µg busereline synthetic analogue gonadotrophin realizing FSH and LH. Females from Group II were intramuscularly inoculated with 0,2 ml Bioreline Biochef containing 0.90 µg busereline synthetic analogue hypothalamic GnRH. The third Group was given 0,2 ml *Aqua pro injectione* (by Polfa, Cracow,). To estimate morphological and structural changes stimulated by used hormonal preparations, females from Group I and from Group

III were anesthetized in the eleventh day and females of Group II– in the eighth day after inoculation. The number of mature and maturing follicles as well as of post-ovulation corpus luteum on ovaries were recorded. In stage two of the experiment, another 24 females were inoculated with the same preparations (as those of stage one) and then housed with a male in a polygamous system, in ratio 1 male to 4 females. This stage was conducted for 6 months. The effect of hormones applying was measured by detection of the number of barren females and the number of kittens born. Fecundity might happen in the first or/and second estrus cycle.

Photo 1 Diestrus phase. Vaginal smear consists mainly of the leukocytes (10x10)



Before hormonal stimulation, the state and phase of reproductive system of females were determined - vaginal smears were collected with the use of sterile slightly curette moistened with distilled water. The smears were then quickly transferred to a slide, fixed in 96% alcohol and ether solution (1:1 v/v) over at least 30 minutes, and then stained with Papanicolau differential method. The share of cells of each epithelium layer, of estrus cornified cells as well as the presence of leucocytes were later determined (PHOTO 1). The number of leucocytes was evaluated under a microscope by a 5-grade range as follows: ++++ mass number of leucocytes, +++ great number of leucocytes, ++ numerous leucocytes, + single leucocytes, - no leukocytes in the field of vision.

Results

The ovary in sexually mature females chinchilla undergo periodical morphological and functional changes. The main function of this progressive process is to release egg cells (ovulation) together with hormonal substances, which alternately generate estrus and stimulate uterine epithelium to receive fertilized oocytes. These changes depend on hormonal activity of hypophysial-ovary axis which itself is a negative feedback [Szoltys, 1992].

Experimental stage one

Both, growing and mature follicles, as well as corpus luteum, were found in ovaries of experimental chinchilla females in the both experimental groups of animals. The number of follicles corresponded to the hormonal preparation. In females from Group I great number of maturing follicles, 6-10 mature follicles on each ovary in particular females and *corpus luteum* in 5 females only were found (TABLE 1). In Group II also large number of maturing follicles, 2-10 mature follicles and *corpus luteum* in all females were found (TABLE 1).In the control Group III, there great number of maturing follicles and 1-3 mature follicles in particular females were observed. No *corpus luteum* were found (TABLE 1).

Table 1 Ovary size and the stage of folliculogenesis in experimental and control chinchillas

Group	Number of females	Ovary length		Ovary dimension		Maturing follicles	Mature follicles	Corpus luteum
		Left	Right	Left	Right			
I	8	6.14 ± 0.42	5.79 ± 0.63	2.95 ± 0.11	3.65 ± 0.66	Numerous	6 - 10	0 - 4
II	8	6,99 ± 1.63	6.21 ± 0.82	4.00 ± 0.35	3.30 ± 0.28	Numerous	2 - 10	1 – 5
III	8	5.27 ± 0.97	5.01 ± 0.34	2.22 ± 0,32	2.56 ± 0.60	Numerous	1 - 3	0

Experimental stage two
Ability of females to fecundation was evaluated based on conception ratio and the number of newborn kits.

Table 2 The results of reproduction of female chinchillas after hormonal stimulation

Group	Number of females	Whelped females	Litter size
I	8	5	1.25 (0-2)
II	8	8	1.6 (1 – 3)
III	8	0	0

In Group I (the animals with Receptol acting on their ovaries), the 5 females were successfully mated and the most of them gave 2 kittens in a litter. In Group II (Bioreline inoculated), all females had kittens: 4 of them gave 1 kitten and the rest had 2 or 3 kittens in a litter. In the control Group, no kittens were born during the trial (Table 2).

Discussion

The GnRH hormone, being synthesized in subthalamic nucleus and released from there, modulates the secretion of gonadotrophines from secretion cells of gland part of hypophysis. The influence of hypothalamus realized by release of LH-RF and FSH-RF [Szoltys, 1990]. There is a high correlation between GnRH pulses and release of LH and FSH, which depends on light [Bielanski & Tischner, 1996]. Primary and secondary follicles on ovaries are stimulated to grow by FSH hormone and, when passing into more complex phase, they secrete estrogens. The follicle phase, intensified by physiological growth of estradiol in blood, stimulates the synthesis process of GnRH receptors and its releasing as well. The GnRH wave must grow rapidly to start generation of LH wave from hypophysis. Additionally, stimulation of GnRH is necessary during the whole period of LH wave [Karsch et al., 1997].

Conclusions

According to morphological and reproduction results, both hormonal substances used in experiment: Bioreline and Receptal stimulate the estrus and ovulation processes in experimental chinchillas ovaries. Both preparations generated rapid growth of growing follicles, (of) ovulation process and production of corpus luteum. However, Bioreline gave better results than Receptol. The litters obtained in the trial also confirm the expectations of application of GnRH analogues. However, Bioreline gave better results than Receptol (more kids were born)

References

Bielanski A., Tischner M., Biotechnologia rozrodu zwierzat gospodarskich, Universitas, Krakow, 1996 (In Polish).

Bromsel-Helmerein O., Hugan L.V., Duran-Gssslin J. The effect of varying doses of FSH on the ovulation and of preovulatory rabbit follicles and oocytes. Human Reproduction, 4, 636-639, 1989

Jarosz S.: Badania nad przebiegiem cyklu plciowego u szynszyli w warunkach klimatycznych Polski, Zeszyty Naukowe WSR w Krakowie, Rozprawy, 17, 1969. (In Polish. English summary)

Jarosz S., Rzewska E.: Chow i hodowla szynszyli. PWRiL, Warszawa, 1996 (In Polish)

Karsch F.J., Bowen J.M. Caraty A., Evans N.P., Moenter S.M. Gonadotropin - Releasing Hormone Requirements for Ovulation. Biology of Reproduction. 56, 303-309, 1997.

Kramer M., Oddzialywanie hormonow gonadotropowych na narzady rozrodcze owcy. Roczniki Naukowe Zootechniki, 7, 2-15, 1980 (In Polish, English summary)

Szoltys M., Struktrura i funkcja pęcherzyków jajnikowych ssaków, Postępy Biologii Biologii Komórki, 19, 3, 221-238, 1992. (In Polish, English summary)

Szoltys M.: Owulacji i supeowulacja w aspekcie morfologicznym i hormonalnym. Endokrynologia Polska, 41, 107-109, 1990. (In Polish, English summary)

Weir B.J.: Aspect of ovulation in Chinchilla. Zoological Society of Reproduction and Fertility, 12, 410-411, 1966.

Wilk S.: Rozrod w fermie szynszyli. Hodowca Drobnego Inwentarza, 3, 10-11, 1989. (In Polish)

IV – 19 RP

Growth parameters and organ size of American marten (*Martes americana*) born in captivity

H. A. Collins, K. Rouvinen-Watt, J. Grant and M. Rankin
Canadian Centre for Fur Animal Research, Department of Plant and Animal Sciences, Nova Scotia Agricultural College, Truro, Nova Scotia, Canada
Email: krouvinen@nsac.ns.ca

Abstract
This research examined the seasonal growth parameters and organ size of juvenile and adult male and female marten (*Martes americana*) born in captivity. The body weight data was collected from 25 male and 20 female marten from the Nova Scotia Agricultural College marten colony during 1999-2003 and the organ weight data from 19 males and 13 females in January 2003 and 2004. At 7 days of age the body weight of the male kits was 61.7±2.1 g and the female kits 55.1±1.7 g (P=0.019), while in December the juvenile males weighed 924.4±17.5 g and the females 633.9±8.1 g (P<0.001). The body weights of the juvenile female marten 631.2±7.9 g were significantly different in January from those of the mature female marten 667.6±11.8g (P=0.013), and the weights of the juvenile males 909.3±16.2 g differed significantly from those of adult males in January 1057.2±29.7 g (P=0.001). The marten exhibit pronounced seasonal fluctuation in their body condition throughout the year with both males and females being the heaviest in April, males being the smallest in August and the females being the smallest in July. Significant sex differences were observed in the weights of most internal organs. These results are valuable for the characterization of growth and seasonal changes in body condition in the American marten.

Introduction
The American (pine) marten (*Martes americana*) is a small fur bearing animal approximately the size of a small house cat with a long and slender body, and is a member of the Mustelidae family (Strickland *et al.*, 1982). The American marten shows sexual dimorphism, with the males being larger and heavier than the females. In wild populations, in Ontario, winter body weights of adult male marten are on average 821 g, and the winter body weights of juvenile male marten 734 g (Strickland & Douglas, 1999). Winter body weights of adult female marten average 482 g, and the winter body weights of juvenile female marten 488 g (Strickland & Douglas, 1999). The body length of a mature male marten is about 50-63 cm, which is longer than the body length of a mature female marten (46-56 cm) (Marshall, 2001). Marten also show visible seasonal variations in their pelt colour and quality. The winter pelt is dark brownish in colour with a distinctive orange-buff patch on the throat (Cornish, 2002).
The American marten is an arboreal species, which prefers dense coniferous forests with sufficient ground litter such as dead trees, branches and leaves to support and provide cover to various rodents, their principal food source (Snyder, 1991). In the wild, marten are opportunistic feeders that hunt mainly on the ground and their diet consists of a variety of items depending on what is available, including mice, voles, snowshoe hares, shrews, red squirrels, birds, fish, eggs, carrion, insects, fruit or nuts (Wydeven, 2000).
Female marten have induced ovulation and delayed implantation of the blastocyst for seven to eight months. The gestation period is 27 days with parturition occurring in late March to early April. There are typically 2-3 kits per litter, each weighing 28 g on average. They are born altricial and thus depend entirely on their mother for food and protection. The kits grow rapidly and by four weeks of age males weigh about 200 g and females about 173 g (Strickland *et al.*, 1982). The kits are weaned at 6 weeks of age, reach full growth by three months and are sexually mature at 15 months of age, but typically do not successfully reproduce until 24 months of age (Strickland *et al.*, 1982). According to a study on the European pine marten (*Martes martes*) both males and females show seasonal fluctuations in body weight,

where the weights are highest in the summer and lowest in the winter (Korhonen *et al.*, 1995). Korhonen *et al.* (1995) also reported that the European pine marten shows sexual dimorphism in some organ weights and sizes.

Since 1990, the Nova Scotia Agricultural College has developed a captive breeding program for the American pine marten (Rouvinen-Watt *et al.*, 1999) with successful matings and whelpings since 1995. The wild species status of the American pine marten has shown a decline in population in the Atlantic Provinces due to excessive trapping for their valuable fur along with accidental trapping (Boss, 1987). In Nova Scotia, the American marten is now a “species at risk”, defined as any indigenous species, variety, or geographically defined population of wild fauna or flora that is at risk of becoming any of the following: extinct, extirpated, endangered, threatened or special concern (COSEWIC, 2002).

Growth parameters such as developmental and seasonal growth curves and organ sizes of the American pine marten (*Martes americana*) born in captivity are not well characterized. The objectives of this research were to create growth curves for captive-born juvenile male and female marten, to establish seasonal body weight curves for adult male and female marten and to measure organ size. This information would be valuable for the evaluation of animal performance in captive breeding and species conservation programs.

Material and Methods

The daily and seasonal care of the American pine marten colony at the Nova Scotia Agricultural College followed the practices outlined in the Standard Operating Procedure for the American marten (Rouvinen *et al.*, 1999). Body weights of 25 male and 20 female captive-born marten were recorded monthly during 1999-2003. The marten were considered juvenile if they were less than one year old and adult thereafter. Marten selected for pelting and subsequent organ scaling data collection consisted of 19 males and 13 females. These marten were euthanised by means of carbon monoxide. Each marten was weighed (g), body length measured (cm), pelted and carefully dissected removing mesentery tissue and fat. The following organs were individually weighed: heart, liver, stomach (full and empty), spleen, pancreas, intestine (full and empty), kidneys, adrenal glands, testes from the male marten and the reproductive tract of the female marten.

For statistical analyses, the marten were grouped into categories of juvenile males, juvenile females, adult males and adult females. For each category, the mean monthly weights (±SD) were calculated. A 2-sample t-test was used to test differences between male and female marten as well as juvenile and adult animals (Minitab Statistical Software). The means (±SD) were calculated for the weight, body length and organ weight data from the euthanised marten. A 2-sample t-test was used to compare if significant differences existed between the male and female marten.

Results and Discussion

Figure 1 represents the growth rate of juvenile male versus female marten born in captivity from 7 days of age to 9 months of age. At 7 days of age the body weights of the male kits were 61.7±2.1 g and the female kits 55.1±1.7 g ($P=0.019$). Both sexes reached their peak body weights in October, when the males weighed 968.4±17.7 g and the females 659.8±9.3 g ($P<0.001$). By December both male and female juvenile marten had reached their adult body size, males weighing 924.4±17.5 g and females 633.9±8.1 g ($P<0.001$).

The male and female marten born in captivity have lower body weights at four weeks of age 156.1±3.3 g and 139.5±3.1 g, compared to body weights reported in literature for wild male and female marten at the same age 200 g and 173 g (Strickland *et al.*, 1982). Winter body weights of captive-born juvenile male (924.4±17.5 g) and female (633.9±8.1 g) marten were heavier, compared to the winter body weights reported in literature of wild juvenile male marten (734 g) and female marten (488 g) (Strickland & Douglas, 1999).

Figure 1. Growth curves for juvenile male and female American marten.

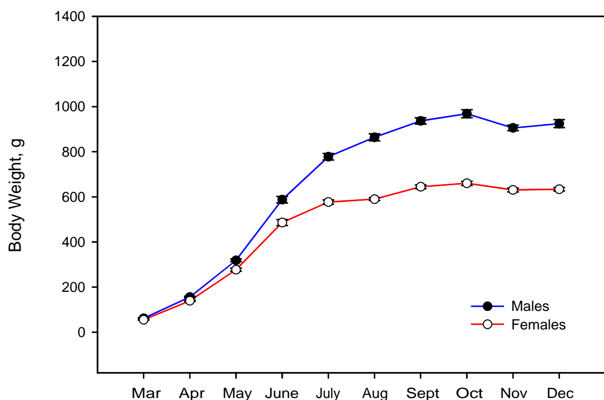


Figure 2. Seasonal fluctuations in body weight of adult and juvenile male and female American marten.

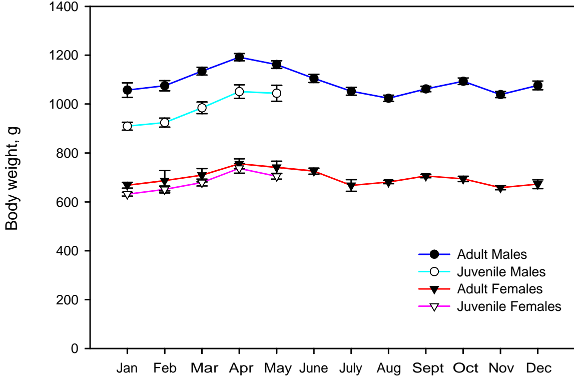


Figure 2 shows the seasonal fluctuations in body weight of adult and juvenile male and female marten. Juvenile male and female marten were included in the seasonal growth curve, but were separate from adults from January to May because they were considered to be fully grown, but not mature. In June, weights of the juvenile male and female marten were incorporated into the adult seasonal growth curves. The body weights of the juvenile female marten (631.2±7.9 g) were significantly different in January from those of the adult female marten (667.6±11.8 g; P=0.013), and the weights of the juvenile males 909.3±16.2 g differed significantly from those of adult males in January (1057.2±29.7 g; P=0.001). Both male and female marten exhibited pronounced seasonal fluctuation in their body condition throughout the year with both juvenile and adult males (1050.9±28.0 and 1191.9±14.8 g) and juvenile and adult females (737.0±19.9 and 755.9±20.6 g) being heaviest in April, adult males being the smallest in August (1023.6±13.2 g) and the adult females being the smallest in July (666.9±23.9 g). It was found that the winter body weights of adult male (1057.2±29.7 g) and female (667.6±11.8 g) captive-born marten were higher than the values reported in the literature for the American marten in the wild with adult males weighing on average 821 g and females weighing 482 g (Strickland & Douglas, 1999). These seasonal fluctuations in body condition for captive-born male and female marten are somewhat different from those reported for the wild European pine marten. Korhonen *et al.* (1995) reported that both male and female European pine marten show seasonal changes in body weight, where the weights are highest in the summer and lowest in the winter due to their limited body fat reserves.

Table 1 shows the comparison of body and organ weights of captive-born male and female marten. All organs, except for the stomach (full) and the spleen (P=0.085), showed a significant difference between the male and female marten. These findings agree with those by Korhonen *et al.* (1995) on organ weights of the wild European pine marten (*Martes martes*) showing large sex differences in the weights of most internal organs. The organ weights of the captive-born American marten appear smaller than the values reported for the European marten (Korhonen *et al.*, 1995) with the exception of the males' spleen and kidneys, and the females' liver and kidneys.

Table 1. Comparison of body and organ weights (mean ± SD) of captive born male and female American marten.

Variable measured	Males	Females	P-value
Number, n	19	13	-
Body weight, g	969±138	651±45	< 0.001
Body length, cm	40.8±1.7	36.0±1.6	< 0.001
Liver, g	44.4±6.3	34.9±2.7	< 0.001
Spleen, g	2.3±1.4	1.6±0.3	0.085
Pancreas, g	2.5±0.3	2.1±0.4	0.002
Stomach (full), g	16.0±11.5	14.2±8.1	0.614
Stomach (empty), g	6.5±0.7	4.7±0.6	< 0.001
Intestine (full), g	30.1±5.9	25.0±5.8	0.023
Intestine (empty), g	18.3±3.8	13.9±2.1	< 0.001
Heart, g	7.2±0.7	5.1±0.5	< 0.001
Kidney (left), g	3.7±0.5	2.9±0.4	< 0.001
Kidney (right), g	3.7±0.5	2.9±0.4	< 0.001
Adrenal gland (left), mg	26.6±3.9	22.4±2.5	0.003
Adrenal gland (right), mg	22.7±3.3	19.8±3.2	0.020
Testicles, g	0.29±0.07	-	-
Reproductive tract, g	-	0.70±0.4	-

Table 2 displays the results from the comparison of organ weights of captive-born male and female marten in relation to body size. Although this method of comparison removes the effect of the large difference in body size due to sexual dimorphism, some significant differences remained between the male and female marten organ weights. In relation to body size, the liver, pancreas, intestinal weight, kidneys and the adrenal glands were relatively larger in the female marten in comparison to the males.

Table 2. Comparison of body and organ weights (mean ± SD) of captive born male and female American marten in relation to body size (% of body weight).

Variable measured	Males	Females	P-value
Number, n	19	13	-
Liver, %	4.6±0.6	5.4±0.3	< 0.001
Spleen, %	0.25±0.17	0.25±0.05	0.970
Pancreas, %	0.26±0.04	0.32±0.05	< 0.001
Stomach (full), %	1.6±1.1	2.2±1.2	0.198
Stomach (empty), %	0.68±0.07	0.71±0.08	0.167
Intestine (full), %	3.2±0.7	3.9±1.0	0.040
Intestine (empty), %	1.9±0.4	2.2±0.3	0.040
Heart, %	0.75±0.07	0.78±0.06	0.188
Kidneys, %	0.78±0.1	0.89±0.07	< 0.001
Adrenal glands, % x 10^{-3}	5.4±0.8	6.5±0.9	0.002
Testicles, %	0.03±0.01	-	-
Reproductive tract, %	-	0.11±0.06	-

Juvenile male and female marten born in captivity show successful developmental growth with a significant difference between sexes. Adult captive-born male and female marten are sexually dimorphic and exhibit pronounced seasonal fluctuations in their body condition throughout the year with males being heavier than the females. Comparisons of adult male and female marten born in captivity showed a

significant difference in body weight, length and the weight of some organs. These results are valuable for the characterization of growth and seasonal changes in body condition in the American marten and can be used to evaluate growth performance of martens in captive breeding and species conservation programs.

Acknowledgements
We thank all staff and students involved in the marten breeding program during 1999-2004, with a special thank you to Rae MacInnis and Rick Russell for their skilful technical assistance.

References

Boss, J. 1987. American Marten Back in Nova Scotia. Conservation. 11 (3). http:/198.166.215.5/natr/WILDLIFE/conserva/11-03-9.htm. [Accessed 6/4/02]

Chapin, T. G., Harrison, D. J. and Phillips, D. M. 1997. Seasonal habitat selection by marten in an untrapped forest preserve. Journal of Wildlife Management 61(3): 707-715.

Cornish, J. 2002. The Newfoundland pine marten: An endangered species. www.stemnet.nf.ca/CITE/newfoundlandia.htm. [Accessed August 18, 2002].

COSEWIC, 2002. Canadian Species at Risk, May 2002. Committee on the Status of Endangered Wildlife in Canada. 34pp.

Ellis, E.J. 1996. *Martes americana*: American Marten, American Pine Marten. University of Michigan. http://animaldiversity.ummz.umich.edu/accounts/martes/m._americana$narrative.html [Accessed June 4, 2002].

Grant, J. and Hawley, A. 1996. Some observations on the mating behaviour of captive American pine martens *Martes americana*. Acta Theriologica 41 (4): 439-442.

Heath, J.P., McKay, D.W., Pitcher, M.O., and Storey, A.E. 2001. Changes in the reproductive behaviour of the endangered Newfoundland marten (*Martes americana atrata*): implication for captive breeding programs. Canadian Journal of Zoology 79: 149-153.

Korhonen, H., Pyyvaara, P., and Niemelä, P. 1995. Energy economy and activity in farmed pine martens (*Martes martes*). Scientifur 19 (4): 270-276.

Marshall, M. 2001. Pine Marten. http://www.regina.k12.nf.ca/grassroots/default.htm [Accessed June 4, 2002].

Rouvinen, K., Rankin, M. and Grant, J. 1999. Breeding and Management of American Marten (*Martes americana*). Standard Operating Procedure. Fur Unit, Nova Scotia Agricultural College, Truro, Nova Scotia, Canada.

Rouvinen-Watt, K., MacRae, C., Patterson, D., Rankin, M. and Grant, J. 1999. Captive breeding of the American pine marten (*Martes americana*). Nordic Association of Agricultural Scientists (NJF). Seminar No. 308. Reykjavik, Iceland.

Snyder, S.A. 1991. Biological data and habitat requirements: *Martes americana*. United States Department of Agriculture. www.fs.fed.us/database/feis/animals/mammal/maam/all.html. [Accessed June 4, 2002].

Strickland, M.A. and Douglas, S. W. 1999. Marten. Pages 531-546 in Wild Furbearer Management and Conservation in North America.(Eds. M. Novak, J. A. baker, M. E. Obbard and B. Malloch). CD-Rom version. Ontario Fur Managers Federation, Ontario Ministry of Natural Resources. Queens Printer, Ontario, Canada.

Strickland, M.A, Douglas, S. W., Novak, M. and Hunziger, N.P. 1982. Marten (*Martes americana*). Pages 599-612 in Wild Mammals of North America Biology, Management Economics. (Eds. J. A. Chapman and G. A. Feldhamer). The John Hopkins University Press Baltimore.

Wydeven, A. P. 2000. Pine Marten (*Martes americana*). Wisconsin Department of Natural Resources. http://www.dnr.state.wi.us/org/land/er/factsheet/mammals/Marten.html. [Accessed November 14, 2002].

SCIENTIFUR
ISSN 0105-2403
Vol. 28, No. 3

Proceedings of the VIII International Scientific Congress in Fur Animal Production

V: Fur Properties

Edited by:

Dr. Bert Urlings
Prof. Dr. Berry Spruijt
Dr. Marko Ruis
Ing. Louise Boekhorst

V – 2 RP

The influence of pelting time on pelt characteristics in blue fox (*Alopex lagopus*)

Leena Blomstedt[1], Lauri Jauhiainen[2], Maija Miettinen[1] and Kerstin Smeds[1]
[1]Finnish Fur Breeders' Association, P.O. Box 5, FIN-01601, Finland
[2]MTT (Agrifood Research Finland), Information Services, FIN-31600, Finland
leena.blomstedt@stkl-fpf.fi

Abstract
Blue fox pelt characteristics were studied in relation to three pelting days (November 20, December 2 and 16) in order to find the optimal pelting time. The study included 720 young blue fox males originating from five farms and from three pelting groups on each farm. Samples were taken from the hip region of dried raw skins for leather thickness and weight measurements. The dried raw skins were graded for fur auction and auction data was collected. The data was analysed by using Friedman's non-parametric test and analysis of variance taking into account litter and farm effects. Fur quality showed no statistically significant difference between the three different pelting dates (p=0,33) although the share of the best fur quality (Saga Royal) increased from the first to the last pelting date (6%, 8%, and 12%). Likewise the share of skins with low and sparse under wool (flat) diminished significantly towards later pelting (16%, 13%, and 7%, $p<0,01$). A larger amount of woolly skins with short and weak guard hair appeared if pelting was postponed ($p<0,01$). The clarity of the fur colour, from bluish to brownish shade, was best in the earliest and poorest in the latest pelting group (the share of most bluish pelts, R+ and R: 47%, 32%, and 32%). The difference between the two first pelting dates was significant ($p<0,001$). The leather became significantly ($p<0,001$) thinner and lighter towards mid-December indicating the maturation of the skin and the fur. The results confirm that the fur volume, especially fur length, still increase in December. Finally the economic balance between the positive price effect of fur density and the negative effects of increasing woolliness and deteriorating colour clarity will define the optimum pelting time.

Introduction
Pelting of blue fox is usually started around mid-November and finished in early December. The features that signal the fur maturity and thus the correct pelting time are e.g. fluffiness and colour of fur and length of under hair. In practise, however, the date of first auction often sets the starting of pelting. Furthermore, a general belief that unwanted fur features start to appear shortly after the traditional pelting time has encouraged early pelting. An earlier pilot study has shown that about 40 % of under hairs in blue fox are still growing and unprime in late November (Blomstedt et al., 2001). An ideal time to pelt would be when there is an optimal balance between the fur maturity and the desired fur characteristics.
The skins in the pilot experiment were studied as dressed and lacked the auction data or other information of economically important fur and leather characteristics. Therefore it was important to perform an experiment, where these relations could be studied. The purpose of our present field study, therefore, was to monitor the priming of blue fox skin from mid-November until mid-December and to analyse possible changes in the skin features and pelt traits in relation to pelting time.

Materials and methods

Animals and pelting.
The animal material was selected from 5 private farms. On each farm 3 equal groups, A, B and C, were formed of male cubs deriving from litters with at least 3 male cubs and born between May 10 and June 5. Each litter was thus represented with one cub in each group. The total material consisted of 720 animals. The feeding and care of animals was according to normal farm practice. On each farm the groups were pelted according to the following time schedule: Group A on November 20, group B on December 2 and group C on December 16. After euthanizing the foxes were skinned and the fresh skins stored in a deep freezer at -20°C. At a later time all skins were thawed, and then fleshed and handled according to common practice on the respective farm.
Raw skin biopsies: From each dried raw skin a biopsy sample (Ø 6 mm) was taken from the site anatomically corresponding to the hip region. Hair was carefully removed and the remaining leather

samples were weighed (g) with an analyse balance and the leather thickness (mm) measured with a digimatic caliper (Mitutoyo).

Pelt characteristics.
The weight (g) of dried raw skin was recorded. Fur characteristics, the coverage of guard hairs and the density of under fur hairs, were graded by a professional skin grader at Finnish Fur Sales Co Ltd (FFS), (scores 1-10, 10 best). Furthermore, the pelts were submitted to the commercial auction grading of the FFS. In this procedure fur colour, colour clarity and pelt size are measured objectively by using the automated sorting and measuring equipments (ATE-Applied Engineering Ltd, Finland 2003) based on very high resolution and high performance detection line scan cameras. Pelt size is measured in centimetres and sorted to major size groups, the difference between two groups being 90 mm. The commercial grading categories for size are: class 50: > 133 cm, class 40: 124,1-133,0 cm, class 30: 115,1-124,0 cm. class 20: ≤115 cm). The fur colour is expressed in pixels (the higher the number the lighter the pelt colour).The colour clarity grades are: R+ (bluish, the best shade); R; R-; OC (brownish, the poorest). Fur quality is classified manually by professional skin graders. The categories for basic fur quality are: SR (Saga Royal=best); S (Saga); I (Quality I); II (Quality II=lowest); III (Quality III=skins with various defects). Quality groups SR, S, I and II are considered as regular skins, quality III as low grades. Of additional quality descriptions following are included in this report: heavy skins (HEAV, very dense under wool, a positive character), woolliness (WOL, short, weak guard hairs, three grades: 1=slight, 3=serious), flat skins (FLAT, low and sparse under wool), bites or damaged hair (CHIP). All pelts from farms 2-4 were sold at the auction in April 2003, all pelts from farm 1 at the auction in December 2003. The skin price is recorded as euros.

Statistics.
The statistical analyses were carried out at the MTT, Information Services. Continuous variables were analysed by using ANOVA taking into account treatment, litter and farm effects (Littell et al., 1996, pages 76-86). Categorical variables measured in ordinal scale were analysed using the Friedman’s non-parametric test, where litter was used as a block effect (Gibbons et al., 1992). Other categorical variables were analysed using the chi-square test for contingency table. All statistical analyses were performed using the SAS software (release 8.2) MIXED and FREQ–procedures (SAS, 1999) and NPARRCB–macro (Berry,1997).

Results and discussion

Pelt size and leather characteristics.
The skins from 3 pelting groups covering a period of 4 weeks did not differ significantly regarding the mean skin weight (Table 1). The majority of the skins in all pelting groups measured 124 – 133 cm (category 40) and represented 49,4%, 45,9% and 47,8% of all pelts in the respective group. Corresponding figures for size group 50 representing the longest skins were 38,6%, 43,7% and 41,8% and for size 30 group 11,6%, 10,4% and 9,9% in pelting groups A, B and C, respectively. In the shortest size group (20) there were only 2 skins. The distribution of all skins sold by FFS in April 2003 auction was: class 50: 19%; class 40: 40%; class 30: 32 % and class 20 or smaller: 7% (Smeds, Sampo program skin information).
In leather samples the average thickness decreased from 0,53 mm to 0,43 mm (s.e.± 0,008) and the average weight from 13,3 mg to 11,6 mg (s.e. ± 0,2) as the pelting was delayed (Table 1).

Table 1. Skin weight, skin length (= size), and leather thickness

		Pelting groups				
		November 20 A	December 2 B	December 16 C		
Dried skin:		**mean**	**mean**	**mean**	**± s.e.**	***p***
Mean weight g		893	896	900	± 7.0	0.56
Mean length cm		134	134	135	± 0.4	0.38
Distribution of skins in size categories						
		A	**B**	**C**		
Size group	**Range in cm**					
Size 50	> 133.0	38.6 %	43.7 %	41.8 %		
Size 40	124.1 – 133.0	49.4 %	45.9 %	47.8 %		
Size 30	115.1 – 124.0	11.6 %	10.4 %	9.9 %		
Size 20	106.1 – 115.0					
Leather samples: ∅ 6 mm		**A**	**B**	**C**		
		mean	**mean**	**mean**	**± s.e.**	***p***
Sample weight (leather) mg		13.3	12.3	11.6	0.0002	< 0.001
Leather thickness mm		0.53	0.47	0.43	0.008	< 0.001

Both changes were significant (p<0,001) and indicate progressing maturation of the leather. Kondo & Nishiumi (1991) have shown that in mink during hair growth the leather part is thick due to a loosely arranged network of collagen bundles and large inter-bundle spaces embedded in a gel like matrix. After the hair growth has ceased, i.e. the fur is mature, the leather part is thin because collagen bundles are tightly packed and the inter-bundle spaces are minimal. A variation in leather sample weight and thickness between skins from the same pelting date may indicate genetic differences as documented in mink (Berg and Lohi, 1991) or differences in the skin structure caused e.g. by diet (blue fox, Dahlman & Blomstedt, 2000). In this study leather samples from longer skins were significantly heavier and thicker than those from shorter skins (class 50: 12,9 mg, 0,50 mm; class 40: 12,2 mg, 0,47 mm; class 30: 11,7 mg, 0,46 mm; class 20: 10,1 mg, 0,41 mm; p<0,005). Connection between animal size and leather thickness (large individual – thick skin, small individual – thin skin) has also been demonstrated in other species, e.g. mink (Harri & al., 1984).

Fur characteristics.

The *coverage of guard hairs* deteriorated significantly from group A to C (scores 7,3; 6,7 and 6,6; scale 1-10 (10 best), p=0,03). On the other hand, the *density of under fur* hairs increased significantly (p<0,001) along time (respective scores: A 6,4; B 6,4 and C 6,7). Guard hairs of blue fox pelage mature around mid-November, while a number of under fur hairs are still growing in length. The total number of under hairs is reached around early November (Blomstedt, 1998) but according to three earlier studies the proportion of mature under fur hairs was on December 1 still only 70-90% (Joutsenlahti et al., 1988, Blomstedt, 1998, Dahlman & Blomstedt, 2000). Thus denser appearance of fur coat at a later pelting is mainly due to the lengthening of under fur as the hairs are priming. How much the characteristics of under fur hairs affect negatively the coverage of guard hairs depends on the length relation and the number of both hair types in the fur coat.

The *fur colour*, measured as pixels, became significantly lighter towards later pelting date (Table 2).

Table 2. Fur colour and clarity of colour

	Pelting groups				
	November 20 A	**December 2 B**	**December 16 C**		
Fur colour:	**mean**	**mean**	**mean**	**± s.e.**	***p***
pixels	347	358	362	± 3.7	< 0.005
Distribution of skins in categories of colour clarity					
	A	**B**	**C**		
Clarity group:					
R + (most bluish, best)	2.1 %	0.4 %	0.4 %		
R	44.6 %	32.0 %	31.5 %		
R -	39.8 %	39.8 %	44.8 %		
OC (brownish)	13.7 %	27.7 %	23.3 %		

The explanation is again found in the growth of the fair coloured under fur hairs that mix with the normally darker guard hairs thus diluting the impression of the fur colour. The *clarity of colour* (categories R+, R, R- and OC) deteriorated significantly (p< 0,001) along the time (Table 2). The main change in clarity distribution occurred between November 20 and December 2 as the proportion of off-coloured (OC), slightly brownish pelts increased from 13,7% to 27,7% (Table 2). A simple reason for this change is obviously the fur getting easily slightly dirty, especially if the weather is humid.

Fur quality of regular skins. Fur quality depends on the density, length and quality of under fur hairs and guard hairs. The distribution of regular skins in quality categories (SR, S, I and II) showed no statistically significant differences (p= 0.33) between the three pelting dates even though the share of the best quality skins (SR) increased towards later pelting (groups: A 6,1%, B 8,1% and C 11,9%). Most skins from all pelting groups were of quality S (A 64,6%, B 65,1% and C 62,2%) and about 25% of quality I (A: 29,2%; B: 26,8%; C:25,4%). Only one skin originating from the latest pelting represented the lowest group of regular qualities (II). The additional positive quality description *heavy* (HEAV) stands for very dense under fur hair. Heavy type increased somewhat towards December without no further increase in the latest pelting group (A 0,0%, B 2,2% and C 1,3%; p= 0,09).

Fur defects. Flat skin (FLAT) with sparse and low under fur belongs to negative additional quality descriptions. The decrease in the share of FLAT skins from group A to group C (19,3%, 14,6% and 7,3%) was statistically significant (p< 0,01), and indicates improved quality. The number of skins with *bites* or damaged hair (CHIP) increased slightly, yet not significantly, towards later pelting (A 5,9%, B 7,8% and C 11,9%; p= 0,11). Possibly the foxes reared in pairs, while getting older, tolerate each other less and nipping of the neighbour becomes more frequent as the time goes by. *Woolly* (WOL) is an expression for short weak guard hairs mainly in the middle part of the back. The poor coverage of guard hairs on the woolly area is more clear the denser and longer the under fur hair is. Thus it is understandable that later pelting makes the defect more visible (Blomstedt & Joutsenlahti, 1987). The defect has genetic background (Lohi, personal communication). The increase in the share of woolly pelts was equal in all grades of the defect (1= slight, 3= serious). Altogether the defect increased significantly from group A to group C (35,7%, 42,8% and 48,9%; p= 0,02).

There was no correlation between fur quality and thickness or weight of leather samples.

Skin price.

The difference of about 4 weeks in the pelting time had no clear effect on the pelt price. The mean price of all skins from the experiment was in group A 52,2, in group B 52,8 and in group C 52,5 euros (p= 0.69).

Conclusions

- Maturation process of the skin part continued linearly throughout the whole experiment. Mature leather is an advantage in manufacturing especially when leather-out garments are produced.
- Hair volume increased from the second half of November until mid-December through length growth of under fur hairs. Thus the amount of skins in the best quality group (SR) and skins with additional quality description heavy (HEAV) increased. Consequently, the amount of flat skins decreased.
- The coverage of guard hair was affected slightly in negative direction. Along with this the proportion of skins marked as woolly (WOL) increased.
- There were more bites in skins pelted on December 16. Possibly rearing foxes in female-male pairs instead of two males in the same cage as in this experiment might diminish these damages.
- Fur colour becomes paler if pelting is postponed. In this experiment the clarity of colour deteriorated slightly after November 20. However, fur colour and colour clarity have very little influence on skin price.
- In this experiment pelting time did not affect pelt price.
- Considering both the fur and the leather part of the skin it seems that the best result is achieved by pelting in early December.

Acknowledgements

Product manager M. Viljanen deserves our thanks for enlightening information and for manual grading of the skins. We wish to thank M.Sci.Agr. O. Lohi for her helpful and valuable comments during the preparation of the manuscript. This study was supported by Helve Foundation and the work was carried out in co-operation with Finnish Fur Breeders’ Association and Division of Animal Physiology, Helsinki University, Finland.

References

ATE-Applied Engineering Ltd, Finland, 2003. Automated blue fox colour shading. European Integrated Machine Vision 2:14-15.

Berry J.J., 1997. The simulation approach to analyzing the nonparametric randomized complete block design. Observations 1(4): 36-44.

Berg P. & Lohi O., 1991. Hair length and leather thickness in mink. Genetic and phenotypic sources of variation. NJF-Utredning/Rapporter nr. 70: 59-67

Blomstedt L, 1998. Pelage cycle in blue fox (*Alopex lagopus*): A comparison between animals born early and late in the season. Acta Agric. Scand., Sect. A, Animal Sci. 48:122-128.

Blomstedt L. & Joutsenlahti U., 1987. Blårävens ullighet. NJF-Utredning/Rapporter nr. 47, (NJFs pelsdyrseminar , Tromsø, Norge, 28.-30. Sept.): 51-54.

Blomstedt L., Miettinen M. & Pölönen I., 2001. Blue fox – pelt characteristics related to pelting date. NJF- seminar no. 331, Sneckersten, Denmark, 1-3 October, 7 pp.

Dahlman T. & Blomstedt L., 2000: Effect of feed protein level on fur and skin of the blue fox. Scientifur 24(4):13-16.

Gibbons J. &, Chakraborti S., 1992. Nonparametric statistical inference. New York, NY : Marcel Dekker 131 pp.

Harri M., Jokivartio K. & Karjalainen M., 1984. Studies on thickened dermal structures of mink. 3rd Int. Sci. Congr. in Fur Anim. Prod., 25-27 Apr., Versailles, France

Joutsenlahti U., Blomstedt L. & Valkosalo K., 1988. Giant blue-fox - new mutation. In: Murphy, B. D. & Hunter D. B. (eds). Biology, Pathology and Genetics of Fur Bearing Animals. Proc. of the 4th Int. Cong. in Fur Anim. Prod. August 21-24, Rexdale, Ontario, Canada. pp: 564-569.

Kondo K. & Nishiumi T., 1991. The pelage development in young mink (*Mustela vison*). J. Fac. Agric. Hokkaido Univ. 64: 247-255.

Littell R., Milliken G., Stroup W. & Wolfinger R., 1996. SAS System for Mixed Models (pp. 76-86), Cary, NC: SAS Institute Inc., 633 pp.

SAS Institute Inc., 1999. SAS/STAT User’s Guide, Version 8, Cary, NC: SAS Institute Inc. 3809 pp.

V – 3 RP

Population genetics and registration of fox pelts in warehouses.

(Khlebnikov’s travel notebooks revisited in terms of the *Hardy-Weinberg low* by Borodin)

O.V. Trapezov

Institute Cytology & Genetics, Siberian Department, Academy of Sciences of Russia; 630090, Novosibirsk, RUSSIA. Fax: 7 (3832) 33 12 78; Tel: 7 (3832) 33 05 12.

E-mail: trap@philosophy.nsc.ru (priv), E-mail: trapezov@bionet.nsc.ru (work)

Abstract

Khlebnikov known as the “*Chronicler of Russian America*” in 1824 filled his “*Special Notebook*” with descriptions of the peltry of *Alaskan black*, *cross*, and *red foxes*, in Alaska and the neighboring islands trapped for commercial purposes. Relying on Khlebnikov’s reports, in 1981 geneticist *Borodin* estimated the number of pelts by method of population genetics is known as *Hardy-Weinberg*’s *law*. This estimation provided answers to puzzling questions: 1) Are the differences in natural viability between *black*, *red*, and *cross* foxes? 2) Did the higher market price of black and cross fur make hunting for foxes carrying the *B* gene preferable? (In fact, a black fox was three times more expensive than a red one at that time). 3) How honest were of the Russian American Company employees involved in pelt production in 1824? What if they biased their data to conceal theft of the exceptionally expensive pelts?

Introduction

August 3, 1784, in the then Russian America, in the *Kodiak island*, the famous merchant of fur *Shelikhov* established the first Russian settlement, in the Three Saints Harbor; June 8, 1799, on the initiative of Irkutsk merchants and with government support, all Russian fur merchants united into The Russian-American Company (RAC).

In 1806, the czar Alexander I approved the flag of the RAC, with beige, lilac, red stripes, and a double-headed black eagle holding a ribbon with the inscription “The Russian-American Company”.

In November 1817, a new governor of the RAC office arrived. This was K.T.Khlebnikov, who became a corresponding member of the St. Petersburg Academy of Sciences. The directorial board of the RAC appointed him manager of the RAC, whose office was located in the island of Sitka (from indian: “Shitha-qwan”) in Russian America. These were the most prolific years of his life. Khlebnikov wrote his “*Notes on California*” published in 1829. As a researcher, he made a collection of what grew and thrived about – ethnographic, entomologic, mineralogic specimens – and sent them to the St. Petersburg Russian Academy of Sciences starting from 1831 (*Shur*, 1972, 1974). November 1832, Khlebnikov left Russian America to occupy an administrative post in St. Petersburg. In 1835, he was elected as a director of the RAC. Khlebnikov has been justly called the “*Chronicler of Russian America*”.

G.I.Shelikhov 1747-1795

T.Khlebnikov 1784 – 1838

Material and Methods

Khlebnikov filled his "*Special Notebook*" with descriptions of the peltry in Alaska and the neighboring islands. He believed that his long residence in the Sitkha island compelled him to write memories about the terrestrial animals, he noted the widely spread *Alaskan black*, *red* and *cross* (or "*sivodushka*", or "*zamarajka*" in Russian) foxes.

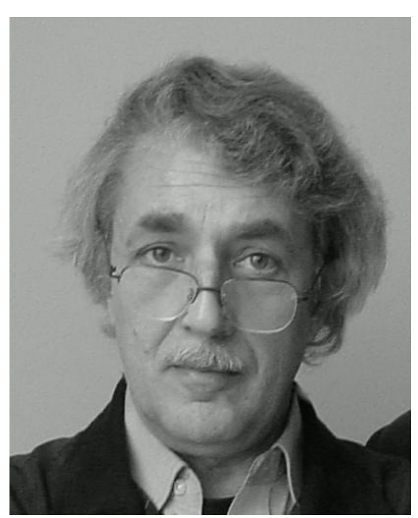
Years later, *Iljina* (the first russian investigator in genetics of coat color in foxes) in her "*Genetics and Selection of Fur Animals*" (Moscow, 1935) observed that populations of foxes inhabiting Kamchatka, Chukotka, Yakutia, Alaska show polymorphism for coat color that is controlled by two alleles of the *B* gene. The *Alaskan homozygous black foxes* are *BB*, while those *homozygous red foxes* are *bb*. Heterozygotes *Bb* showing intermediate coat color are called *cross foxes* ("*sivodushka*", "*zamarayka*", "*bastard*" in Russian) (*Iljina*, 1935).

E.D.Iljina (1910-1986)

Turning away from *Khlebnikov*'s "*Special Notebook*", we find that in 1824 trapped by hunters for commercial purposes in the Kodiak island were 59 *black foxes*, 104 *cross foxes*, 89 coarse coat *red foxes*, and there followed a list of fox wild-caught at various locations of the island (*Liapunova & Teodorova*, 1979).

D.K.Belyaev (1917-1985).
The eminent russian geneticist in the field of fox breeding

P.M.Borodin (born 1948).
A follower of D.K.Belyaev



A hundred fifty years elapsed, when in 1981 the young researcher at the Institute of Cytology and Genetics (Novosibirsk, Russia) *P.M.Borodin*, a follower of the *eminent fox breeder D.K.Belyaev*, realized the implications of Khlebnikov's reports and narratives. "*What*, Borodin wondered, *would they mean, after all*? *And then he had it! Khlebnikov's reports were, in actual fact, most detailed gene-geographical descriptions of fox populations occurring in Russian America*" (*Borodin*, 1981; *Borodin*, 1982).

Results and Discussion

Relying on Khlebnikov's reports, Borodin estimated the number of pelts of *black* and *cross* foxes (i.e., the concentration of the *B* gene) in population that was distributed in the area neighboring the Three Saints Harbor.

Borodin also relied on *Iljina's data* according to which coat color of *Alaskan black fox* is controlled by two mutant genes (*BB*). *Red foxes* do not have the *B* gene and, therefore, their genotype is *bb*. As for heterozygous *cross foxes*, every one carries a *B* gene and a *b* gene. Borodin calculated the frequency of the *B* gene in the fox population at The Three Saints Harbor which in those years was:

$$\frac{2 \times 59 + 104}{2 \times (59 + 104 + 89)} = 0{,}44$$

What did the calculations disclose? They provided answers to puzzling questions: 1) Were there differences in viability between *black*, *red*, and *cross* foxes, that is to say, were foxes subject to selection for this character (color phase)?; 2) Did the higher market price of black and cross fur make hunting for foxes carrying the *B* gene preferable? (In fact, a black fox was three times more expensive than a red one at that time). 3) How honest were the RAC employees involved in pelt production in 1842? What if they biased their data to conceal theft of the exceptionally expensive pelts?

Borodin made the following calculations to clarify the issues. Based on the list of pelts, Borodin calculated that the frequency of the *B* gene in the Three Saints Harbor population was 0.44. In the same way the *b* gene was calculated as 0.56. This meets expectation, if the entire composition of the fox population is expressed as 1 (100%), the concentration of the *B* allele as *p*, and the concentration of the *b* allele as $1-p$. In fact, the alternative states (two alleles) of a gene are under consideration.

At the given gene frequencies, what should be the number of *black*, *red*, and *cross* foxes in the Three Saints Harbor population? To give an answer, Borodin referred to the mathematical law of the variability in coat color in a wild population of fur bearing animals. In population genetics, the law is known as *Hardy-Weinberg*'s (after the English mathematician *Hardy* and the German physician *Weinberg*).

How did this law come into being?
Why do eminent animal breeders still give it due consideration?

The answer goes far back. In 1908, at the Cambridge University, after supper, the geneticist *Pennet* and his friend *Hardy*, both gifted talkers, exchanged ideas about the science already in vogue, genetics, of course. Pennet stated that he has heard critical comments on Mendel's theory and that he was groping for answers.

Thus, for example, if the gene for short fingers would be dominant, and the one for long fingers would be recessive, then the number of individuals with short fingers would keep increasing from generation to generation. After several generations, there would no long fingers in Great Britain?!

Pennet did not share this line of reasoning, but he could not explain why it was incorrect. In response Hardy said that the explanation was a cinch – sufficed a couple of formulas written straight away on his napkin. On the spot Hardy proved, to Pennett's surprise, that, at a definite frequency of genes for normal and short fingers, the relative number of individuals with long or short fingers remains the same in each generation in the absence of natural or artificial selection, or differential migration, or other factors affecting gene frequency. Hardy thought that the conclusions were rather trivial, not worthy of being published. However, Pennet insisted that the conclusions deserved a better treatment than a napkin inscription. By that time, the German physician *Weinberg* independently arrived at the same conclusion. And accordingly the law became known as the *Hardy-Weinberg law.*

In fact, the same rule was earlier stated by the geneticist *Castle*, who collaborated with the mink breeder *Moore* and contributed much to the genetics of coat color in mink and horse (*Castle & Moore,* 1946; *Castle,* 1948). However, Castle is not mentioned in textbooks with reference to this law.

The *concept of Hardy-Weinberg* crystallized from this law. According to the concept the frequencies of alleles became the foundation of theoretical population genetics.

It also heralded the coming into being of genetics (Mendelism) as an independent science and its establishing alliance, or synthesis, with the theory of evolution. Subsequently, the Russian biologist *Chetverikov* (1880-1895) and his followers paved the way to population genetics a

basis to any consideration of any evolutionary event.

The *Hardy-Weinberg* law in population genetics may be equated to Newton's first law of motion in mechanics, which states that a body remains in its state of rest or uniform motion unless acted on by external forces. The *Hardy-Weinberg* law states that, in the absence of disturbing ecological processes, gene frequencies will be retained unaltered. However, in real ecological settings, processes disturbing gene frequencies are continually at work.

It is well to remind, at this juncture, that the *Hardy-Weinberg law* has been applied with reference to natural fox populations by *Iljina* and *Romashov*. They have published the relevant materials in a paper entitled "*Analyses of fox populations by Hardy*'s *formula*" in 1942 (*Romashov & Iljina*, 1942).

In the case considered here, the *B* allele is *codominant*, or *incompletely dominant*, to the *b* allele. Matings of foxes of two genotypes *BB* × *bb* yields an F_1 progeny with foxes of the *Bb* – genotype (*cross foxes*), with mixed pelage containing hair of color inherited from both the black and the red foxes. Calculation of gene frequencies in matings between the F_1, individuals demonstrated that a quarter of the population in the F_2 will be of the *BB* genotype, a half of the *Bb* genotype, and the genotype will be *bb* in the remaining quarter.

To determine the proportion of the F_3 that will be the progeny, say, of matings between individuals of *BB* × *Bb*, genotypes, let us multiply ¼ by ½, the product is ⅛. Thus, provided mating is random, ⅛ of the population will be the offspring of this pair. The question is, what proportion of the generation will be offspring of the pairs *BB* × *BB* ? *Bb* × *bb* ? All these combinations will yield ⅛ of all the number of individuals of the next generation F_3. Then, what proportion of it will be heterozygous *cross* foxes *Bb* ? Having calculated the frequencies of gametes of this type among the possible combinations, 4 × ⅛ = ½ is obtained. What is the number of individuals of genotype *Bb* in the F_2 generation? Also half. What if we calculate the results of all the possible combinations in the F_3 generation and in any next generation? It proves that the ratio of the genetic components in a population are retained unaltered!

Conformance with the *Hardy-Weinberg equilibrium* in application to the *black*, *red*, and *cross* foxes in *Khlebnikov*'s *records* requires satisfaction of the following conditions. 1) The fox population must be composed of a very large number of individuals; 2) Matings between the different color phase foxes must be entirely random; 3) The male and female sex cells, or gametes, carrying the alleles *B* and *b* must unite at the time of fertilization entirely at random to form the zygote, an elementary organism starts to develop. In such a case, the appearance probability of a zygote of each type, under random mating in the given population is determined only by the frequencies of the *B* and *b* genes. In this equation, *p* is the frequency of the *B* gene; *q* is that of the *b* gene; p^2, $2pq$ and q^2 are the phenotype frequencies of the *black*, *red*, and *cross* foxes, respectively.

In random mating, the equilibrium frequencies of the genotypes are expressed as the products of the corresponding alleles. In the examined case, there are only 2 alleles, *B* and *b*, with frequencies *p* and *q*. For this reason, the frequencies of the three possible genotypes will be written as

$$(p + q)^2 = p^2 + 2pq + q^2 = 1.$$

In this way, *Borodin* calculated that in conformance with the *Hardy-Weinberg law* one would theoretically expect for the Three Saints Harbor population in 1824 that the frequencies of alleles for any character in a population in any generation would remain unaltered provided no external disturbing effects intervene. In the given case, the allele *B* is *codominant* or *incompletely dominant* to the *b* allele. Mating between foxes of two genotypes *BB* × *bb* will yield an F_1 generation of *Bb* genotype – with coat having intermingled black and red hair. Calculation of the frequencies of genes in matings between individuals of the F_1 generation discloses that ¼ of the population will be of the *BB* genotype in the F_2 , ½ of the *Bb*, and ¼ of the *bb* genotype. The quadratic equation familiar to all the high schoolchildren underlies the *Hardy-Weinberg law.* According to the law, in application to the *black*, *red*, and *cross* foxes in *Khlebnikov*'s *records*, the equation for coat color of foxes for future sale would be written as

$$p^2BB + 2pqBb + q^2bb = 1$$

In conformance with the *Hardy-Weinberg law*, one should theoretically expect the following estimates for the trapped foxes in populations that was distributed in the area neighboring the Three Saints Harbor in 1824: 49 *black*, 124 *cross*, 79 *red*.

From the records in *Khlebnikov*’s “*Special Notebook*”, it follows that there were 10 times more *black* and *red* foxes than theoretically expected, the number of *cross* was 20 times smaller than expectation. Do these differences between the expected and the observed number of trapped foxes suffice to regard the calculated assumptions valid for the Three Saints Harbor fox population? Statistical tests demonstrated that the differences were due to random causes.
From the equation it further follows that throughout the year foxes showed no preferential mating to either *red*, or *black*, or *cross* foxes.
From *Khlebnikov*’s *notes* Borodin, made another very important inference, namely hunting was not targeted at foxes of a particular coat color (whatever *black*, *red*, or *cross*). This was because foxes were mainly trapped.
What about the morale of the professional hunters and buyers of pelts at the RAC? A fair deal was lucrative for the partners, with one supplying, and the other buying <u>*all*</u> the pelts. Really, it cannot be imagined that the professional hunters sold off pelts to foreign merchants without sorting them by color. They did sort them. Table 1 gives the price list of fox pelts of different colors in roubles in the bygone days (*Slunin*, 1895, 1900).
From the prices listed in Table 1, it follows that it would be more profitable to steal pelts of *black* than *cross foxes*, and theft of *cross* would be more profitable than of *red*. <u>In the case of preferential theft of color phases, the *Hardy-Weinberg law* would be necessarily violated</u>. Since in *Borodin*’s calculations there was conformance with the *Hardy-Weinberg law* at virtually all the fur loading stations in Russian America. The honesty of pelt business at the Russian-American Company must be acknowledged.
Nevertheless *Borodin* noted that gross deviations from the *Hardy-Weinberg law* did occur at the trading stations. He detected them in materials recorded in the trading stations located in the islands of *Unga* and *Nuchek* where the “*Bostonians*” (thus *Khlebnikov* called merchants from the United States) “*played their pranks*”. It is quite conceivable that it was not a matter of honesty, control of the RAC at the other trading stations; the simple reason was absence of buyers (no “*Bostonians*”).
Accusations, moreover hasty, became pointless after the lapse of centuries. What if the culprits of the deviation from the *Hardy-Weinberg* gene frequencies were fox populations themselves, and the human factor was irrelevant? The islands are small and so were the fox populations inhabiting them. *It should be reemphasized that that the Hardy-Weinberg law concerns only populations of small size*!
This clears up matters. In fact, there was a considerable deficiency of heterozygotes (*cross foxes*) that certainly arose as a result of decrease in population size and inbreeding. *Borodin* also thought that this inference was justified on the basis of the estimates made by *Khlebnikov*, the “Chronicler of Russian America”.

Table 1. The price list of fox pelts of different color in roubles.

Color phase	Years						
	1801	1827	1836	1850	1890	1891	1896
Red	0,4	2	2	3	4-5	3-5	5
Cross	0,8	3	4	6	8-10	8-12	8-15
Black	2	6	6	9	80	60	150

References

Borodin P.M. 1981. Phenotype and gene frequencies in red fox populations of Russian America in 1803-1832. J. Heredity, v. 72, № 5, p. 343.

Borodin P.M. 1983. Stable polymorphism in the red fox populations of Kamchatka. Journ. General Biology, v. 43, p. 541-546 (In Russian).

Castle W.E., Moore L. 1946. Mutations of mink under domestication. J Hered 37:137-145.

Castle W.E. 1948. The ABC of color inheritance in horses. Genetics 33:22-36.

Iljina E.D. 1935. Bases of the Genetics and Breeding of Fur Animals. Moscow, Glavpushnina (In Russian).

Khlebnikov K.T. 1979. Russian America in unpublished notes. Compiled by R.G.Lyapunova and S.G.Fedorova. Leningrad. Nauka. (In Russian).

Romashov D.D. Iljina E.D. 1942. An analysis of fox populations by Hardy’s formula. Comm. USSR Acad. Sci. 37:220-224 (In Russian).

Sljunin N.V. 1895. Hunter wealths in Kamchatka, Sakhalin and the Comandore islands. (In Russian).

Sljunin N.V. 1900. The Okhotsk-Kamchatka territory. (In Russian).

Shur L.N. 1972. Russian scientists and travellers – researchers of the ethnography of California (separate reprint) Bulletin of the Academy of Sciences of the USSR. M., P. 120 (In Russian).

Shur L.N. 1974. Diaries of travels in California (1820-1831) (separate reprint). Proceedings of the All-Union Geographical Society, 106, P. 402 (In Russian).

V – 4 RP

Hair density and morphology of medulla in Mustelidae

Keiji Kondo[1], Yoshitake Ninomiya[1], Hideo Ichikawa[2], Masaru Kato[2], Shigeharu Fukunaga[3] and Asako Kondo Hosaka[1]
[1] Laboratory of pelage diversity, 3-8-10, Matsuba-cho, Kitahiroshima, 061-1136 Japan
[2] Botanic garden museum, Hokkaido University, Sapporo, 060-0003 Japan
[3] Faculty of Agriculture, Hokkaido University, Sapporo, 060-8589 Japan

Abstract
Insulation of pelage depends on its density and length. Hair density is an important factor to determine the quality of fur, and it is also important for mammals to adapt to their habitat. This study was carried out to measure hair density and to observe morphology of medulla in Japanese mustelids. Hair density was calculated from number of hair per hair bundle and number of hair bundle per cm^2. Morphological observations of medulla were made with JSM. The hair density was high in order in sea otter, river otter, mink, ermine, sable, Japanese weasel, least weasel. The morphology of medulla observed with SEM varied more than expected. It was suggested that the observation of medulla with SEM may identify its species. This study suggested the possibility of clarifying whether hair density and morphology of medulla depend on taxonomy or habitat by examining the skins from individuals inhibiting in different environments.

Introduction

Insulation of pelage depends on its volume, that is, its density and length. Therefore, fur breeders have been interested in hair density, as it is an important factor to determine the quality of fur. Hair density of major fur animals is known (Kaplan 1971), while not many studies concerning other mammals than mink (*Mustela vison*) and fox (*Vulpes vulpes*) indicate how to measure the density. The authors of this study made public how to measure hair density (Kondo et al., 1989) and those have been appreciated (Blomstedt, 1992; Nixon, 1993).
Many of the studies on hair density thus far were conducted from the viewpoint of people who use fur for keeping them warm and there have not been many studies conducted from the viewpoint of significance for mammals. This study measured hair density of Japanese mustelids based on the hair density measurement method authors adapted. It also observed the morphological structure of medulla of guard hair which is considered to be related to insulation of pelage as well as to habitat of mammals (Kondo, 2001; Kondo et al., 2002). Finally, it discussed hair density and morphology of medulla in relation to habitat of mammals.

Materials and methods

The winter skin samples used in measuring hair density and observing medulla were collected from the following mammals; sable (*Martes zibellina*), least weasel (*Mustela nivalis*), ermine (*Mustela erminea*), Japanese weasel (*Mustela itatsi*), mink (*Mustela vison*), river otter (*Lutra lutra*), sea otter (*Enhydra lutris*).
Specimens of skins (approx. 1 cm^2) for observation were taken from the dorsal side of each skin close to the tail. The specimens were soaked into detergents for 24 hours, dehydrated by alcohol.

Measurement of hair density

Hair density was determined according to the method applied previously (Kondo et al., 1989). The hair on the specimens cut from the skin samples were first sheared, and then the number of hair bundle (HB) per a unit area (2.5 mm x 2.5 mm) was counted at ten places on the surface of each specimen using a stereo microscope with micrometer. Also, each skin specimen was sectioned perpendicularly to the backbone, and the number of hair (H) per one hair bundle was measured using a scanning electron microscope (SEM). After these

measurements, hair density (number of hair / cm^2, HD) was calculated from the following formula; HD = H x HB / cm^2

Observations of hair medulla

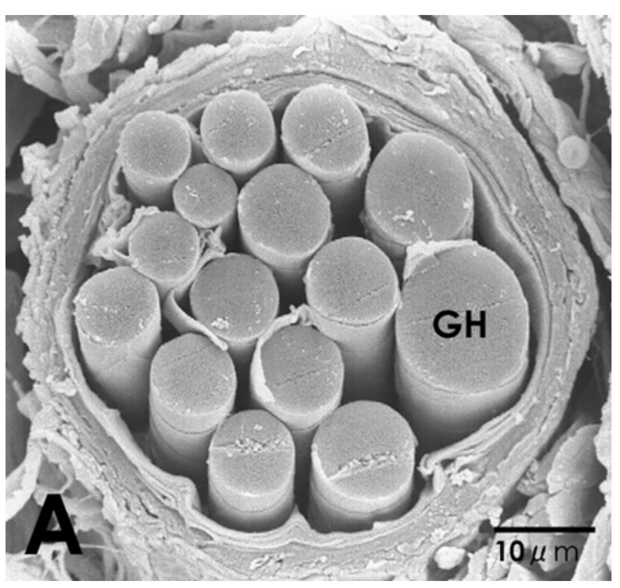
Observations of the hair medulla with SEM were made according to the method applied previously (Kondo et al., 1985). First, guard hairs taken from each fur-skin sample were attached onto brass standard stubs with scotch tape, and then cut along the axis of the fiber using a razor blade under the stereo microscope. The prepared stubs were coated with gold by ion-spattering apparatus. Observations were made with a JSM-T220 SEM at 15kv.

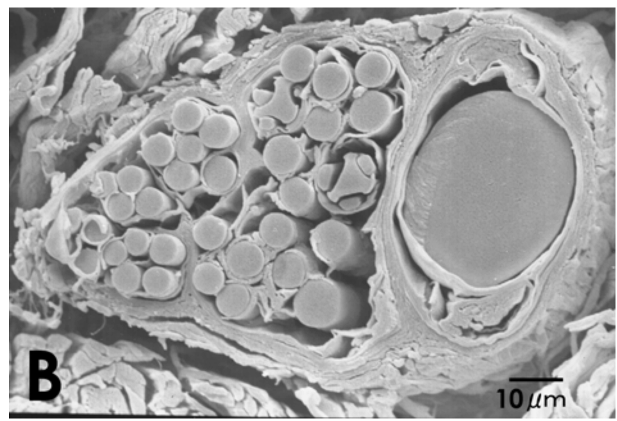
Results

Hair density

The examples of the hair bundle used to calculate the hair density are shown in figure 1, which are the hair bundles of sable and sea otter. In the hair bundle of sable (A), one guard hair and 14 underfurs are shown. In sea otter (B), one guard hair and 36 underfurs.

Fig.1. SEM features of hair bundles.





A: Hair bundle of Sable (Martes zibellina) composed of guard hair and 14 underfurs.
B: Hair bundle of Sea otter (Enhydra lutris) of guard hair and 36 underfurs.

The results of number of hair per hair bundle, number of hair bundle per cm^2 and hair density are shown in table 1.

Table 1. Number of hairs/bundle, number of bundles/cm^2 and hair density

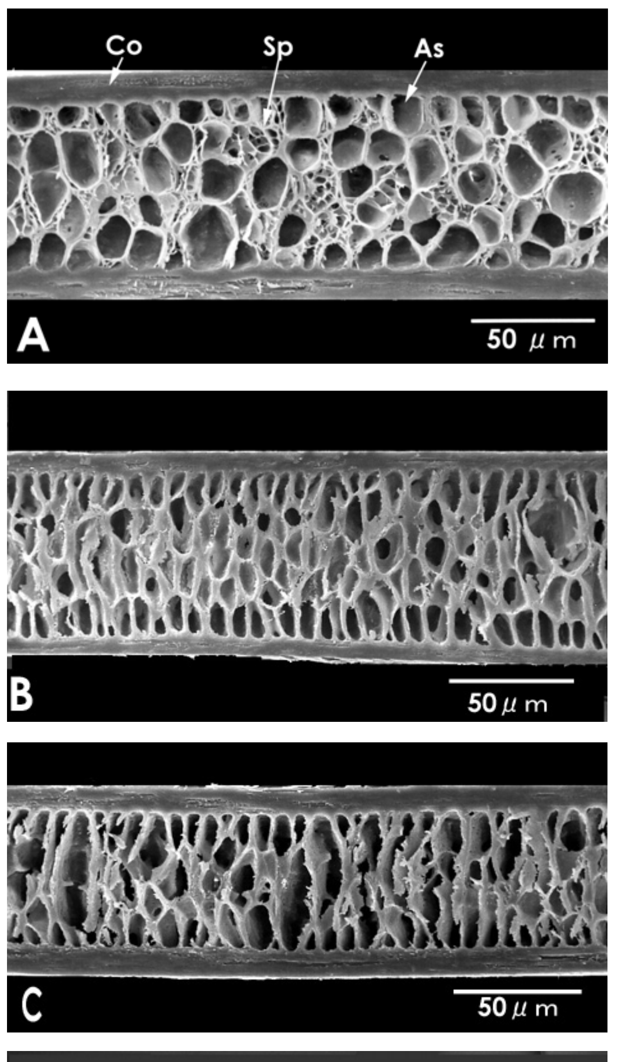
	No.of hairs/bundle X±SE	No. of bundles/cm^2 X±SE	Hair density /cm^2
Sable	12.4±0.38	1,037±39	12,860
Least weasel	8.2±0.29	687±16	5,630
Ermine	12.1±0.40	1,450±40	17,550
Japanese weasel	17.4±0.96	727±21	12,650
Mink	26.3±1.12	1,283±16	33.720
River otter	34.2±0.71	1,714±33	58,600
Sea otter	34.1±0.77	4,520±92	154,100

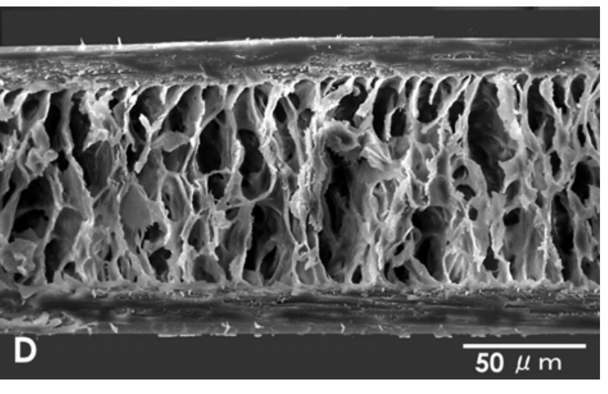
Table 1 shows that the hair density was high in sea otter, river otter and mink. The hair density of sable, ermine and Japanese weasel were much lower than in the first group. Least weasels' hair density was extremely low compared to the other mustelids used in this experiment.

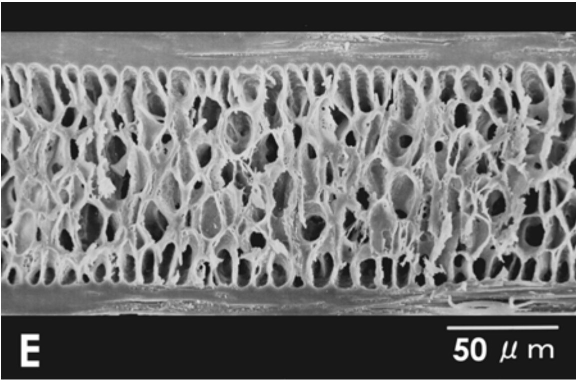
Morphology of Medulla

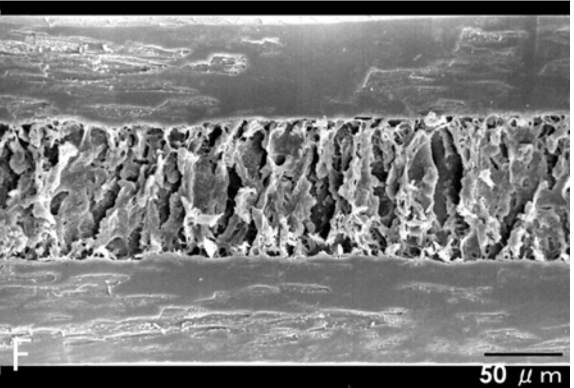
Morphology of medulla observed with SEM is shown in Figure 2.

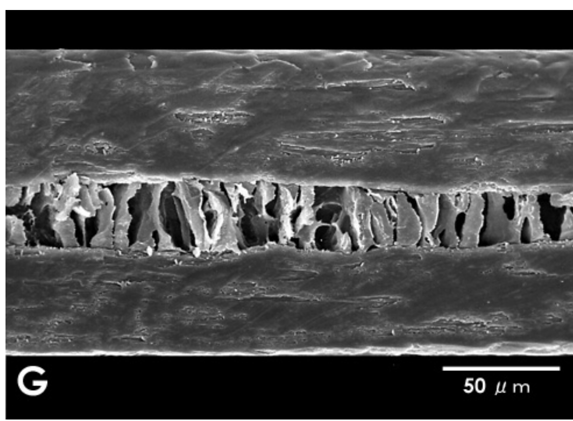
Fig. 2. SEM features of medulla sectioned longitudinally.











A: Sable (Martes zibellina),
B: Ermine (Mustela erminea)
C: Least weasel (Mustela nivalis),
D: Japanese weasel (Mustela itatsi)
E: Mink (Mustela vison)
F: River otter (Lutra lutra),
G: Sea otter (Enhydra lutris).
As: air space, Sp: small air space separated in small cancellus sectors, Co: hair cortex.

In medulla of sables, each air space was the largest found among mustelid samples used in this experiment. These air spaces were mostly circle-like polygon shape or oval. Among these air spaces, small air spaces (Sp) separated in small cancellus sectors were also observed (Figure 2-A).
In ermine, each air space was smaller than that of sables but it was similar in shape, which was circle-like polygon or flat oval. However, small cancellously separated air spaces were not observed (Figure 2-B).
In medulla of least weasel, oval air spaces found in ermine were observed as more flattened shape (Figure 2-C).
In Japanese weasel, air spaces were observed more flattened than least weasels and neither polygon nor oval-shaped air spaces were found. Medulla of Japanese weasels showed rough shapes as a whole (Figure 2-D).
In medulla of minks, polygon-shaped air spaces were observed, not like Japanese weasel. Their shapes were more like those of sables and ermines (Figure 2-E).
Medulla of river otters was much different from those of sables and Japanese weasels and their air spaces were ladder-shaped. Also, it was observed that the proportion of medulla (diameter of medulla / diameter of whole fiber) was lower than in other mustelids except sea otter (Figure 2-F).
Air spaces in medulla of sea otters were much more ladder shape than those of river otters. The proportion of medulla was even lower than in river otters (Figure 2-G).

Discussions

Table 1 shows the hair density depends on the number of hairs per hair bundle and the number of hair bundles per unit area. The samples that showed high density, such as sea otters (approximately 150,000), river otters (approximately 60,000) and minks (approximately 30,000), have many hairs per hair bundle. Therefore, hair density depends more on number of hairs per hair bundle. The number of hairs per hair bundle of ermines was almost as many as that of sables, but hair density was higher in ermine than in sable. The difference of number of bundles per unit area makes hair density of ermines higher than sables. It contradicts the above-mentioned tendency. Future observations including measurement of hair length and proportion of medulla in each hair of ermines and sables are needed to clarify the relationships among insulation, habitat and taxonomy.
Hair density differs between individuals in the same species or between positions in the same individual (Kaszowski et al., 1970; Kondo et al., 1989). Therefore, the hair densities measured in this experiment are not absolute but approximate figures. High hair densities, however, measured in river otter and sea otter may be their characteristics. River otters are the very aquatic in mustelids, which are basically terrestrial mammals. Sea otters are even more aquatic. Therefore, in mustelids, the more aquatic the species is, the higher hair density will be. This may also explain why the higher hair density was observed in minks than sables or other terrestrial species in mustelids.
It is interesting that hair density of least weasel was extremely low among mustelids used in this experiment. Level of hair density reflects mammal' habitat as hairs act as an insulator to maintain their body temperature. This experiment used only one body of least weasel so it may be too early to conclude, but it can be said that the life form of least weasels in winter may be unique among terrestrial mustelids. Comparison between individuals taken from different habitat is expected to verify this, but hair density may be one of the data to surmise the unknown life form of mammals.
The authors have pointed out that the less the proportion of medulla in hair would be, the more aquatic the mammal is (Kondo, 2001; Kondo et al., 2002). As medulla is composed of air spaces, which take in airs, low proportion of the transverse section occupied by medulla means low heat insulation. Therefore, it may be said that the aquatic animals, whose insulation is low because of their hair structure, adapt themselves to the environment with high hair density.
Morphology of medulla is regarded as a key to identify the species just as cuticles, the surface structure of hair (Wildman, 1954; Brunner & Coman,

1974; Teerink, 1991). In the past, studies on morphology of hairs were observed under a light microscope. The authors of this paper used SEM to observe morphology of hairs and clarified that the relationships between mammal classification and the morphology of medulla in guard hair agrees well with the family level (Kondo et al., 1986; Kondo, 2001). Figure 1 shows morphology of medulla varies more than expected. More specifically, in mustelids, morphology of medulla observed with SEM can identify species. In addition, measurement under a light microscope on the proportion of medulla may be useful in identifying hair (Funato, 2002). Therefore, combination of observation with SEM and measurement under a light microscope will increase the accuracy of identification.

Cuticles, which constitute the outermost layer of hairs, are vulnerable to damages by various factors. Therefore, it would be fairly difficult to identify the species by the observation of cuticle when the hairs were stored for a long time, or taken from feces or alimentary canals. On the other hand, medulla, inner component of hair, is not vulnerable to damages and usually maintains their structures. In this respect, observation on morphology of medulla would be useful in identifying hair.

Pelage is important for mammals to adapt themselves to the environment. Even in Japanese mustelids, each species inhibits under various environments. This study suggested the possibility of clarifying whether hair density and medulla depend on taxonomy or habitat by examining multiple skins from different individuals inhibiting in different environment.

References

Blomsted, L. 1992. Pelage growth and structure in fur animals. Norwegian Journal of Agricultural Sciences. Suppl. 9: 577-588.

Brunner, H. and Coman, B., 1974. The identification mammalian hair. Inkata press. Melbourne

Funato, Y. 2001. Study on classification of fur of animals in Mustelidae and their identification. B.A. thesis, Faculty of Agriculture, Hokkaido University.

Kaplan, H., 1971. Furskin processing. Permagan press

Kaszowski, S., Rust, C. C. and Shakelford, R.M. 1970. Determination of hair density in the mink. Journal of mammalogy. 51: 27-34.

Kondo, K. 2001: The diversity of mammalian pelage. Journal of the Faculty of Agriculture, Hokkaido University. 70: 9-17.

Kondo, K., Ninomiya, Y., Ichikawa, H., Kato, M., Shimizu, Y. 2002: Diversity of furskins. *Hikakukagaku*. 48: 1-5.

Kondo, K., Araki, E. ad Ohsugi, T., 1986. Ann observation of the morphology of the medulla in mammalian hairs using a scanning electron microscope. Journal of the mammalogical society of Japan. 10: 115-121.

Kondo, K., Kohno, K., Nishiumi, T., Jin, S. Y., Shimizu, Y., and Ohsugi, T. 1989. Determination of hair density in mink (*Mustela vison*). Scientifur. 13: 15-18.

Nixon, A. J. 1993. A method for determining the activity state of hair follicles. Biotechnic & histochemistry. 68: 316-325.

Teerink, B. J. 1991. Hair of west European mammal. Cambridge University press.

Wildman, A.B., 1954. The microscopy of animal textile fibers. Leeds