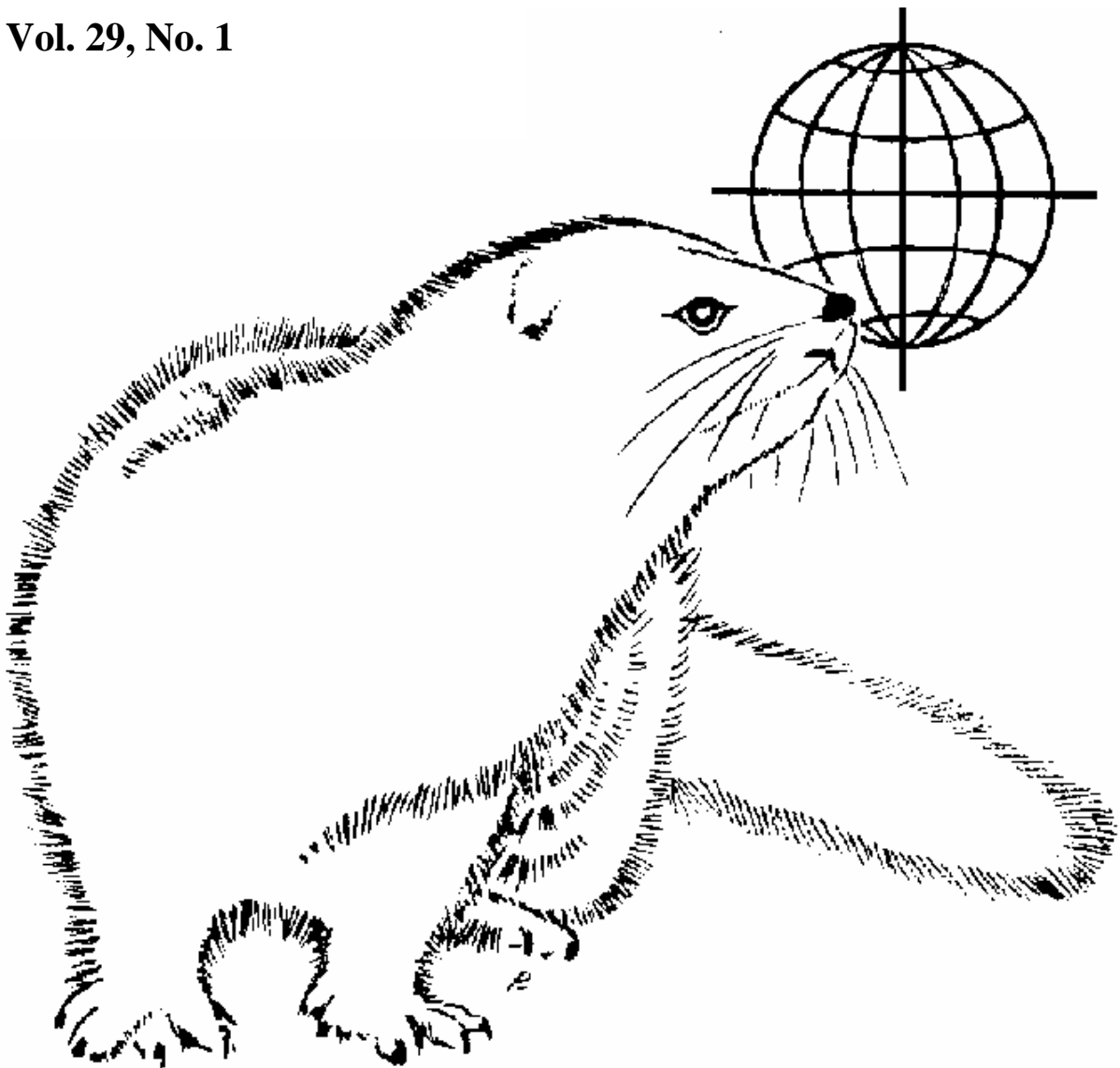


# SCIENTIFUR

SCIENTIFIC INFORMATION IN FUR ANIMAL PRODUCTION

Vol. 29, No. 1



INTERNATIONAL FUR ANIMAL SCIENTIFIC ASSOCIATION

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**Birthe M. Damgaard**  
**SCIENTIFUR**  
**P.O. Box 14**  
**DK-8830 Tjele, Denmark**

**Tel: +45 89991512**  
**Fax: +45 89991500**

**E-mail: [Scientifur@agrsci.dk](mailto:Scientifur@agrsci.dk)**

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**Steen H. Møller**  
**IFASA**  
**P.O. Box 14,**  
**DK-8830 Tjele, Denmark**

**Tel: +45 89991346**  
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**International Fur Animal Scientific Association (IFASA).** Board of directors:

Dr. Bruce D. Murphy (president): E-mail: [murphyb@MEDVET.Umontreal.CA](mailto:murphyb@MEDVET.Umontreal.CA)

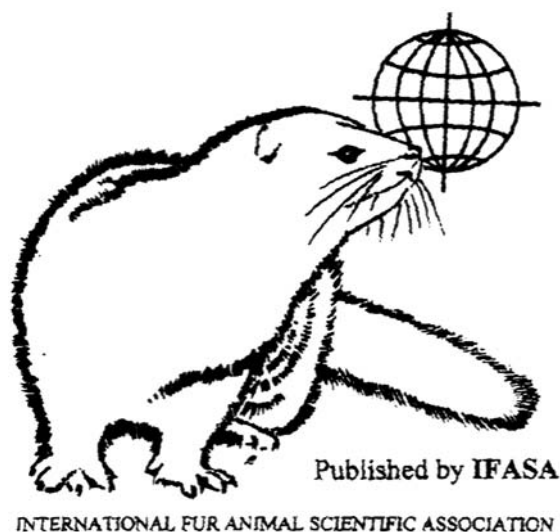
Dr. Steen H. Møller (vicepresident, treasurer): E-mail: [IFASA@agrsci.dk](mailto:IFASA@agrsci.dk)

Dr. Ilpo Pölönen. E-mail: [ilpo.polonen@stkl-fpf.fi](mailto:ilpo.polonen@stkl-fpf.fi)

Ing. Wim Verhagen. E-mail: [info@nfe.nl](mailto:info@nfe.nl)

Dr. Marian Brzozowski. E-mail: [brzozowskim@delta.sggw.waw.pl](mailto:brzozowskim@delta.sggw.waw.pl)

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## Notes from the Group of Editors

This issue of Scientifur, which is the first issue of Volume 29, contains a number of abstracts, including the abstracts on fur animal production presented at a scientific congress in Poland, September 2004. Furthermore, the issue contains the abstracts included in the Annual Report 2004 of the Danish Fur Breeders' Research Center.

For future issues of Scientifur, we invite our readers to submit proceedings, articles for reviewing, short communications, abstracts and letters on fur animal production.

On behalf of the  
Group of Editors

Birthe Damgaard



**Exploitable genetic variation can be changed under environmental and genetic stress. Consequences for livestock breeding - a review**

*H. Kräußlich*

Livestock breeding is exploitation of genetic variation in herdbook populations. Results of long term selection (domestication) experiments led to the assumption that some genes are normally silent and can be activated under genetic and environmental stress. Rutherford and Lindquist (Nature 336: 336–342, 1998) provided the first evidence of a molecular mechanism which can activate silent genes. This article discusses this field of research with respect to the livestock breeding.

*Journal of Animal Breeding and Genetic, 2000: 117, 275.*

**A Marker Set for Construction of a Genetic Map of the Silver Fox (*Vulpes vulpes*)**

*A.V. Kukekova, L.N. Trut, I.N. Oskina, A.V. Kharlamova, S.G. Shikhevich, E.F. Kirkness, G.D. Aguirre, G.M. Acland*

The silver fox, a variant of the red fox (*Vulpes vulpes*), is a close relative of the dog (*Canis familiaris*). Cytogenetic differences and similarities between these species are well understood, but their genomic organizations have not been compared at higher resolution. Differences in their behavior also remain unexplained. Two silver fox strains demonstrating markedly different behavior have been generated at the Institute of Cytology and Genetics of the Russian Academy of Sciences. Foxes selected for tameness are friendly, like domestic dogs, while foxes selected for aggression resist human contact. To refine our understanding of the comparative genomic organization of dogs and foxes, and enable a study of the genetic basis of behavior in these fox strains, we need a meiotic linkage map of the fox. Towards this goal we generated a primary set of fox microsatellite markers. Four hundred canine microsatellites, evenly distributed throughout the canine genome, have been identified that amplify robustly from fox DNA. Polymorphism information content (PIC) values were calculated for a representative subset of these markers and population inbreeding

coefficients were determined for tame and aggressive foxes. To begin to identify fox-specific single nucleotide polymorphisms (SNPs) in genes involved in the neurobiology of behavior, fox and dog orthologs of serotonin 5-HT1A and 5-HT1B receptor genes have been cloned. Sequence comparison of these genes from tame and aggressive foxes reveal several SNPs. The close relationship of the fox and dog enables canine genomic tools to be utilized in developing a fox meiotic map and mapping behavioral traits in the fox.

*Journal of Heredity, 2004: 95, 185-194.*

**Eurasian Otters, *Lutra lutra*, Have a Dominant mtDNA Haplotype From the Iberian Peninsula to Scandinavia**

*A. Ferrando, M. Ponsà, J. Marmi, X. Domingo-Roura*

The Eurasian otter, *Lutra lutra*, has a Palaearctic distribution and has suffered a severe decline throughout Europe during the last century. Previous studies in this and other mustelids have shown reduced levels of variability in mitochondrial DNA, although otter phylogeographic studies were restricted to central-western Europe. In this work we have sequenced 361 bp of the mtDNA control region in 73 individuals from eight countries and added our results to eight sequences available from GenBank and the literature. The range of distribution has been expanded in relation to previous works north towards Scandinavia, east to Russia and Belarus, and south to the Iberian Peninsula. We found a single dominant haplotype in 91.78% of the samples, and six more haplotypes deviating a maximum of two mutations from the dominant haplotype restricted to a single country. Variability was extremely low in western Europe but higher in eastern countries. This, together with the lack of phylogeographical structuring, supports the postglacial recolonization of Europe from a single refugium. The Eurasian otter mtDNA control region has a 220-bp variable minisatellite in Domain III that we sequenced in 29 otters. We found a total of 19 minisatellite haplotypes, but they showed no phylogenetic information.

*Journal of Heredity, 2004: 95,430-435.*

### Coat colour genes in diversity studies

*H. Klungland, H.G. Olsen, M.S. Hassanane, K. Mahrous, D.I. Våge*

In this paper we describe the use of polymorphic genes affecting coat colour as a tool in diversity studies of domestic animals. Although phenotypic data has been the main criteria for establishing different breeds, calculation of genetic distances between breeds is normally performed using noncoding microsatellite markers. As anticipated, *MCI-R* (melanocyte stimulating hormone receptor) allele frequencies vary greatly between cattle breeds expressing different coat colours. In multicoloured breeds, like Icelandic cattle, a high frequency of the  $E^+$  allele appears to be essential for colour variation. Whereas black breeds have a high frequency of the dominant acting allele  $E^D$ , entirely red breeds have no  $E^D$ . Animals being homozygous for the defective allele  $e$  occurred frequently in some cattle breeds, indicating that the *MCI-R* does not have crucial impact on animal physiology other than coat colour. The  $E^+$  and  $e$  alleles were observed in the closely related river buffalo as well. None of the breeds included in this study express the roan phenotype. Consequently, they were monomorphic at the *MGF* locus. As for the *MCI-R* locus, a correlation to colour pattern was observed for two *c-kit* alleles as well, confirming that selection of specific phenotypes strongly affect the allelic variation of underlying loci. Information on genes affecting the phenotype is therefore well suited for describing different breeds of livestock and, consequently, a practical tool in breed conservation.

*Journal of Animal Breeding and Genetics, 2000: 117, 217, 4 tables, 31 refs.*

### Domestication-related variations of the hair cuticula pattern in mammals

*W. Meyer, A. Schnapper, G. Hülmann, H. Seger*

Based on a computer-assisted method and regression analysis, several structural parameters of the hair cuticula pattern of primary hairs of 94 wild mammals and 44 breeds of 10 domesticated mammals were evaluated and compared between both groups. Biologically relevant relationships between the parameters examined could only be

obtained for the wild animals. In this way domestication-induced defects became evident for the hair cuticula pattern that is a typical structural feature of the hair shaft.

*Journal of Animal Breeding and Genetics, 2000: 117, 281, 1 fig, 9 refs.*

### Microsatellite polymorphism and genetic distances between the dog, red fox and arctic fox

*J. Klukowska, T. Strabel, M. Mackowski, M. Switonski*

This study compared polymorphism of nine canine-derived microsatellites (MS) (CPH1, CPH3, CPH6, CPH11, 2004, 2010, 2140, 2168 and 2319) in three species of the family *Canidae*. The DNA samples of 151 dogs, 53 arctic foxes and 91 red foxes were examined. The canine-derived primers did not amplify two MS (CPH1 and CPH11) in genome samples of the arctic fox. The most polymorphic MS in the studied species was the one named 2319. For majority of the loci mean allele size was higher in the dog than in two fox species. The genetic distances between the species were estimated using three formulas:  $D_S$ ,  $D_a$  and  $(\sigma^2)^2$ . The estimated distances between both fox species were the smallest, while those between the dog and the arctic fox were the largest.

*Journal of Animal Breeding and Genetics, 2003: 120, 88.*

### A missense mutation in the follicle stimulating hormone receptor (*FSHR*) gene shows different allele effects on litter size in Chinese Erhualian and German Landrace pigs

*Z. Jiang, O.J. Rottmann, O. Krebs, J. Chen, H. Liu, F. Pirchner*

The follicle stimulating hormone receptor (FSHR) is a unique member of the glycoprotein hormone receptor family. Its expression is highly gonad- and cell-specific, demonstrating its importance for oogenesis and spermatogenesis. Based on published porcine *FSHR* gene sequence, a pair of primers was designed to amplify a 674-bp fragment of exon 10.

The genetic polymorphism was detected by PCR-single strand conformation polymorphism (PCR-SSCP) analysis and a C/T substitution was revealed by direct sequencing of PCR products of putative SSCP homozygotes. This C-to-T transition led to an isoleucine 377-to-threonine substitution in the porcine FSHR protein. Bi-directional PCR amplification of specific allele (Bi-PASA) assay was developed to genotype this C/T substitution on 75 pure Chinese Erhualian (347 litter records) and 113 pure German Landrace sows (501 litter records). The general linear model (GLM) procedure of SAS analysis revealed significant associations of the marker with total piglets born in these two breeds, but showing different allele effects. In Chinese Erhualian pigs, the homozygous T/T genotype was associated with 2.12 and 1.46 piglets more in total born than the homozygous C/C and heterozygous C/T genotype ( $p = 0.0137$ ). In contrast, German Landrace pigs of homozygote T/T produced 1.92 and 2.14 piglets less in total born than pigs of homozygote C/C and heterozygote C/T ( $p = 0.0499$ ). This result indicates that genes could interact differently due to the genetic background in livestock species.

*Journal of Animal Breeding and Genetics, 2002: 119, 335, 3 figs, 1 table, 23 refs.*

### **Mason's World Dictionary of Livestock Breeds, Types and Varieties**

Book review

*Journal of Animal Breeding and Genetics, 2002: 119, 284.*

### **A genetic description of two selected strains of rabbits**

*V. Kerdiles, H. de Rochambeau*

Two strains of rabbits (strains 1077 and 2066) have been selected since 1974 for increased litter size. Each strain is split into a fixed number of reproduction groups. The mating scheme is similar to random non-sib herd mating. The number of animals per generation is higher in strain 1077 (28 mated bucks and 104 mated does) than in strain

2066 (17 and 59, respectively). The increase of the inbreeding coefficient is higher in strain 2066 than in strain 1077 (27 and 21%, respectively, at generation 20). The mean standard deviation of the individual inbreeding coefficients per generation is low (less than 4%) in both strains. Short-term inbreeding is stable over generations. The observed inbreeding effective size  $N_{ef}$  and the observed familial structure effective size  $N_{eh}$  converge to the same value. The number of founder genomes still present in the genetic pool of the generation decreases regularly: from 5.6 to 2.4 between G6 and G20 for strain 1077. It is a bit lower over time for strain 2066 (from 5.7 to 1.9 between G6 and G20). The effective number of founders is nearly twice as small as the total number: 30 for strain 1077 and 15 for strain 2066. The effective number of major ancestors decreases slightly in strain 1077 (13.6 in G6 and 12.9 in G20) and regularly in strain 2066 (13.8 in G6 and 9.4 in G20).

*Journal of Animal Breeding and Genetics, 2002: 119, 25, 2 figs, 4 tables, 23 refs.*

### **A Bayesian analysis of response to selection for uterine capacity in rabbits**

*A. Blasco, J.M. Argente, M.A. Santacreu, D. Sorensen, J.P. Bidanel*

A divergent, eight generation selection experiment on uterine capacity in rabbits was performed. Rabbit does were ovariectomized unilaterally before puberty, and selected for increased and decreased litter size by 'best linear unbiased prediction' using data from up to four parities. Two different analyses were performed to estimate the response to selection. The first was based on least squares analysis; the second was based on Bayesian methods using Gibbs sampling techniques. Three different priors were used for variance components, but these had little influence on the results. Posterior means of heritabilities for uterine capacity, varied from 0.09 to 0.12, and repeatabilities from 0.18 to 0.22. The response to eight generations of selection was symmetrical and led to a divergence of 0.16 young rabbits per generation, which amounts to about 2% of the average litter size of the base population per generation. The pattern of response however, was not linear: a high initial response was followed by a period where little further response

was observed, and a final burst of response was obtained during the last two cycles of selection.

*Journal of Animal Breeding and Genetics, 2001: 118, 93, 1 fig, 4 tables, 21 refs.*

### **A canine linkage map: 39 linkage groups**

*F. Lingaas, T. Aarskaug, J.A. Gerlach, R.K. Juneja, M. Fredholm, J. Sampson, N. Suter, N.G. Holmes, M.M. Binns, E.J. Ryder, W.A. Van Haeringen, P.J. Venta, J.A. Brouillette, V. Yuzbasiyan-Gurkan, A.N. Wilton, P. Bredbacka, M. Koskinen, S. Dunner, D. Parra, S. Schmutz, C. Schelling, J. Schläpfer, G. Dolf*

A low resolution canine marker map is an important tool in the further advancements in genetic analysis of dog breeds and the control and reduction of the frequency of inherited diseases. This study presents a genetic linkage analysis with 39 linkage groups using 222 polymorphic canine markers based on typing in the International DogMap reference families, consisting of 129 Beagle and German Shepherd dogs. Of these 39 linkage groups, 14 have been assigned to canine chromosomes by fluorescence in-situ hybridization (FISH). These results are a further refinement on the first linkage groups from the International DogMap collaboration and represent a continuing collaboration.

*Journal of Animal Breeding and Genetics, 2001: 118, 3, 1 fig, 2 tables, 23 refs.*

### **Variation between ranch blue fox populations in cranial form**

*J. Welling, M. Harri, T. Rekilä, K. Rouvinen-Watt, B.O. Braastad*

The aim of this study was to describe the differences in cranial size and shape that occur between different farmed blue fox populations within and between countries. The skulls were obtained at the pelting time from three Finnish, one Estonian, one Norwegian and seven Canadian farms. The material was subjected to a principal component (PC) analysis for each sex. The first PC-factor explained about 50% of variation. It was identified as the size

factor; it discriminated Finnish blue foxes at one end and Nova Scotia foxes at the other end of the scale. The second PC-factor explained about 10% of variation in skull morphology but failed to discriminate the populations. The third factor, which was dominated by interorbital width, discriminated Nova Scotia foxes from the other populations. PC-factor 4 received its highest loading from the length of upper tooth row. This factor differentiated, although poorly, the Newfoundland fox populations, whether local or crosses between the local and the imported Finnish stock, from the others. The sexes were significantly different on most single parameters and the skulls of all farm populations were larger than those of wild Arctic foxes. However, there were also large differences in skull morphology between farms within one country. This shows that farmed blue foxes in different countries have not yet diverged into anatomically distinct populations.

*Journal of Animal Breeding and Genetics, 2001: 118, 37, 2 figs, 2 tables, 15 refs.*

### **A world dictionary of livestock breeds, types and varieties**

*F. Pirchner*

Book review

*Journal of Animal Breeding and Genetics, 2000: 117, 318.*

### **Effect of capture, immobilization and handling on rectal temperatures of confident and fearful male mink**

*H. Korhonen, S.W. Hansen, J. Malmkvist, B. Houbak*

The objective was to compare response levels of stress-induced hyperthermia (SIH) in male mink from confident and fearful breeding lines. In experiment 1, effects of capture by trap, immobilization and recapture by trap were compared (n = 20 juvenile males per group). Experiment 2 clarified of how capture by trap and repeated temperature measurements in the tight trap

position affected SIH response (n = 9 males, aged 1–2 years per group). In experiment 3, effects of capture by hand and short-term handling in arms on rectal temperatures were evaluated (n = 11 juvenile males per group). Hyperthermic response in the mink was fast. Furthermore, treatments such as capture, recapture, immobilization in a trap and handling in arms were found to evoke measurable temperature responses. After first capture, no significant differences in SIH response between animals of different temperament lines were noted. However, fearful and confident animals tended to react differently to trap immobilization; the fearful ones by increasing or maintaining their response levels, and confident ones by decreasing them. SIH response to recapture by trap was opposite in the temperament groups; after recapture SIH was significantly higher and lower in fearful and confident animals compared with the first capture, respectively. It is concluded that systematic temperament selection has led to significant differences in stress responses between animals selected for confident and timid behaviour. Higher stress reactivity in animals from a fearful temperament line is the most plausible explanation for the lower breeding success observed in previous studies.

*Journal of Animal Breeding and Genetics, 2000: 117, 337, 3 tables, 30 refs.*

### **Estimation of heritability for hip dysplasia in German Shepherd Dogs in Finland**

*M. Leppänen, K. Mäki, J. Juga, H. Saloniemi*

The heritability of hip dysplasia in the German Shepherd Dog was estimated by applying the animal model and the Restricted Maximum Likelihood (REML) method to a data-set which consisted of the hip scores of 10335 dogs. Fixed effects of the model were the month and the year of birth, screening age, the panelist responsible for screening and the origin of the animal's sire. The litter and the breeder had only minor effects on hip joints. Heritability estimates were moderate (0.31–0.35). The moderate heritability, which was found in this study, enables a much better genetic gain in the breeding programme, if proper evaluation methods, such as BLUP animal model, and effective selection is used instead of phenotypic selection.

*Journal of Animal Breeding and Genetics, 2000: 117, 97, 4 tables, 20 refs.*

### **Spontaneous Aleutian disease in a ferret**

*Y. Une, Y. Wakimoto, Y. Nakano, M. Konishi, Y. Nomura*

A 3-year-old female ferret died five days after admission to a veterinary clinic for treatment of acute dyspnea and posterior paresis. Blood chemistry showed no hypergammaglobulinemia. Histopathological examination revealed mild to severe inflammatory infiltrates, composed mostly of plasma cells, in multiple organs. Lesions were especially severe in the kidneys, where focal segmental membranous glomerulopathy was also present. In the liver, in addition to lymphocytic and plasmacytic infiltration in periportal areas, dilatation and proliferation of the bile ducts were seen. On analysis of PCR products, using primers directed against the gene encoding Aleutian disease (AD) viral capsid and formalin-fixed kidney samples, we detected a single band of about 400 bp, specific to the AD virus.

*Journal of Veterinary Medical Science, 2000: 62, 553-555, 5 figs, 11 refs.*

### **Social cognitive evolution in captive foxes is a correlated by-product of experimental domestication**

*B. Hare, I. Plyusnina, N. Ignacio, O. Schepina, A. Stepika, R. Wrangham, L. Trut*

Dogs have an unusual ability for reading human communicative gestures (e.g., pointing) in comparison to either nonhuman primates (including chimpanzees) or wolves. Although this unusual communicative ability seems to have evolved during domestication, it is unclear whether this evolution occurred as a result of direct selection for this ability, as previously hypothesized, or as a correlated by-product of selection against fear and aggression toward humans - as is the case with a number of morphological and physiological changes associated with domestication. We show here that fox kits from an experimental population selectively

bred over 45 years to approach humans fearlessly and nonaggressively (i.e., experimentally domesticated) are not only as skillful as dog puppies in using human gestures but are also more skilled than fox kits from a second, control population not bred for tame behavior (critically, neither population of foxes was ever bred or tested for their ability to use human gestures). These results suggest that sociocognitive evolution has occurred in the experimental foxes, and possibly domestic dogs, as a correlated by-product of selection on systems mediating fear and aggression, and it is likely the observed social cognitive evolution did not require direct selection for improved social cognitive ability.

*Current Biology, 2005: 15, 226-230, 4 figs, 23 refs.*

#### **Effects of diet calcium: phosphorus ratio and metabolizable energy content on development of osteochondrosis, foot bending and performance in blue foxes**

*H.T. Korhonen, M. Happonen, T. Rekilä, J. Valaja, I. Pölönen*

The study evaluates the effects of two dietary Ca : P ratios (1.5 : 1 v. 2.5 : 1) and metabolizable energy (ME) contents (17.3 MJ/kg dry matter (DM) v. 19.2 MJ/kg DM) on the development of osteochondrosis, foot bending and performance in juvenile male blue foxes (*Alopex lagopus*). Four experimental groups (no. = 10 per group) were formed : (1) low energy, low Ca : P (LELC); (2) normal energy, low Ca : P (NELC); (3) low energy, normal Ca : P (LENC); and (4) normal energy, normal Ca : P (NENC). The experiment started at weaning in mid July and finished in early October. From mid August onwards, animals on the normal energy diets (NELC, NENC) grew significantly faster ( $P < 0.001$ ) than animals on the low energy diets (LELC, LENC). The final body weights of the normal energy groups were 1.5 kg higher than those of the low energy groups ( $P < 0.001$ ). The dietary Ca : P ratio did not affect live-weight gain. Foot bending increased significantly from summer to autumn ( $P < 0.001$ ). Changes in foot bending between initial and final evaluations showed that bending was significantly greater ( $P < 0.05$ ) in animals on normal energy (NELC, NENC) than on low energy diets (LELC, LENC). Significant differences were not

found in the mean degree of damage in foot and cartilage between the groups. Ulna breaking strength was significantly higher ( $P < 0.05$ ) in normal energy (NELC, NENC) than in low energy (LELC, LENC) animals. Ulna calcium and phosphorus concentrations of the normal Ca : P (LENC, NENC) groups were significantly higher ( $P < 0.001$ ,  $P < 0.05$ ) than those of the lower Ca : P (LELC, NELC) groups. The bone (ulna) calcium and phosphorus concentrations tended to be higher in the normal energy (NELC, NENC) than in the low energy (LELC, LENC) groups (calcium  $P = 0.07$ ; phosphorus  $P = 0.06$ ). The bone Ca : P ratio was higher ( $P < 0.001$ ) in the normal (LENC, NENC) than in the low Ca : P diet (LELC, NELC) animals. The carcass weights of normal energy animals (NELC, NENC) were significantly higher ( $P < 0.001$ ) than those of low energy (LELC, LENC) animals. The fat : dry matter ratio was higher ( $P < 0.05$ ) in normal (NELC, NENC) than in low energy (LELC, LENC) carcasses. We conclude that the Ca : P ratio of the diet has no effect on the development of osteochondrosis or bending of the foot. The higher body weight caused by normal as opposed to low metabolizable energy content seems, however, to increase the incidence of foot bending.

*Animal Sciences, 2005: 80, 325-331, 4 figs, 3 tables, 36 refs.*



## Scientific Conference on the subject

### 'Animal Breeding'

Poland, September 2004

*Animal Production Review, Applied Science Reports, Warsaw, Poland, 2004,  
Vol 72, No 6 (in Polish)*

Below are the summaries relating to fur animal production.

#### **Impact of high energy feeding on erythrocytic indices in mink (*Mustella vison*)**

*H. Bis-Wencell, L. Saba, A. Kopczewski, B. Likos-Grzesiak, M. Pyzik-Moleda, M. Ondrasovic*

The objective of the studies was to determine the influence of high energy feeding on some blood haematologic indices in minks.

The material for the examination was constituted by blood samples collected from heart of the living minks intended for slaughter from A and B farms situated in the western (A) and southeastern part (B) of Poland. From each farm there were chosen 30 representative individuals. The animals were fed appropriate balanced high-energy feed supplemented with vitamin-mineral premix GuyoFox at a dose 0,1%, and an antioxidant Rendox 200-250 ml/t additive. Till November sodium pyrosulphite was used at a dose making 0.02-0.03% of ready feed mass. The zoohygienic parameters in both farms discussed were equal, the farm A, however, met the European Union requirements concerning the animal hygiene values, feedstuff microbiology and the sanitary conditions of its preparation. The levels of the following direct indices were established: red blood cell count (RBC), haemoglobin concentration (HBG), hematocrit value (PCV) and the indirect indices: mean volume of red blood cell (MCV) as well as mean haemoglobin mass (MCH) and mean haemoglobin concentration (MCHC) in erythrocyte. The obtained results were statistically analysed with two-factor methods. The significant differences were recorded at the levels  $Pf0.05$  and  $Pf0.01$ . The

conducted investigations allowed drawing the following conclusions. No statistically significant differences were recorded in the RBC levels and haemoglobin values. A hematocrit value and MCV level at the minks from the farm A proved to be statistically significantly lower compared to the individuals from the farm B, contrary to MCHC which was statistically significant higher than in the farm A. Statistically significant differences were not reported in case of mean haemoglobin mass in erythrocyte (MCH). Most of the analysed parameters was found within the standards given by Berestov. Some elevated values were recorded only for hematocrit (farm B), while decreased in MCHC levels (farm A and B).

#### **Development of reference values of some leucocytic indices against the high energy feeding of mink (*Mustella vison*)**

*H. Bis-Wencell, L. Saba, A. Kopczewski, M. Pyzik-Moleda, B. Likos-Grzesiak, O. Ondrasovic*

The work aimed at determination the reference leucocytic parameter values subject to a feeding method of minks fed high energy feedstuff. Material for examination was constituted by the blood sample taken from heart of living animals intended for slaughter from a farm situated in the southeastern Poland. From the total group of minks scan brown, 30 representative individuals were chosen and then two treatment groups were made (control and experimental). The minks were fed appropriate balanced high energy feed. The energy value of 1 kg feed ranged from 1700-1690 Kcal/kg depending on the growth and development stage, in that the energy from protein 33.0%, from fat 52.2%

from sugars 14.8% of ready feed. The feed was supplemented with vitamin-mineral premix in such doses that the element requirements were met, i.e. GuyoFox at dose 0.1 kg/t of ready feed. Till November the animals from experimental group were supplied with dietary antioxidant Hadox at amount of 200-250 ml/t and preservative sodium pyrosulphite at amount of 0.2%/t. With analyzer of hematological (type MS 9) leucocyte count was established as well as leucogram. The obtained results were depicted with arithmetic mean ( $\bar{x}$ ) and standard deviation (Sd). The investigations performed allowed to diagnosing leucopenia in the animals of both groups. A leucocyte level appeared to be slightly lower at the animals from the experimental group. The leucogram values developed as follows: lymphocyte and granulocyte counts were lower, while monocytes exhibited a higher level in the animals from the experimental group. A different picture was observed considering percentages of particular leucogram elements. Lymphocytes and monocytes determined in the group with a dietary antioxidant reached higher values, while granulocytes lower, compared to the control group.

### **Reproductive traits' characteristics of Beige chinchilla females**

*R. Brach, J. Bieniek*

Reproductive performance of 30 Beige female chinchillas with 74 deliveries was analyzed. The mean age at the first mating was 390 days. The first, second and third days open were 111.9 days, 67.7 days and 37.3 days, respectively. As much as 37.3% of all pregnancies were the result of postpartum oestrus (up to 2 days post partum), and the matings of the next 36.5% females took place at three subsequent days open (3 to 149 days). The average litter size was 1.85, and the range was from 1 to 4. The 18.2% of the animals were stillborn, and the losses during the rearing period were equal to 3.13%.

### **The influence of adding digest enzymatic preparations on growth and fur quality in common foxes**

*M. Brzozowski, D. Dzierzanowska-Goryn, E. Zakrzewska-Czarnogorska, R. Glogowski*

Enzymatic preparations increasing fiber digestibility are widely used in animal feeding, mainly in poultry and pigs. The enzymatic preparations increase the value of breeding parameters and economical results. There are very little publications concerning the application of enzymatic preparations in carnivorous fur animals feeding. The mixture of three enzymes ( $\alpha$ -amylase,  $\beta$ -glucanase and xylanase), which increase barley and wheat digestibility, was used during the experiment. The result shows that using the mentioned enzymes, when the level of corn in diet reaches 20% did not influence on growth and fur quality in Silver common foxes.

### **Feed consumption and conversion rate of complete mixtures by young coypus during rearing period**

*R. Cholewa, K. Miarka, K. Pawliczak-Maj*

The study was carried out on 79 young Greenland nutrias of both sexes. Two diets with different content of crude protein, 12% (feeding group I) and 17% (feeding group II) were tested. Besides it, both rations in the form of meal and pellets were given *ad libitum* for the animals to choose. The body weight of all animals and feed consumption and conversion were checked on every 4 weeks. The body weight of the nutrias at the final stage of their lives (196 days) was 5345.5 g for males and 4143.1 g for females. The animals in the feeding group II were always heavier than those in the group I. A distinct differentiation between the groups of males, was observed. The highest body weight gain occurred at the beginning of the experiment and later, it was reduced. The maximum value was

achieved already in the first half of the experiment. The animals from the group II reached the highest body weight earlier than those from the group I. Comparison of feeding results in both groups revealed higher consumption and conversion of pellets in the group II which was more pronounced in males than in females. Meal consumption and conversion by males and females was higher only from the 20th week of life in the group II.

### **Coat colour indices and its topographic differentiation of Castorex rabbits**

*R. Cholewa, K. Pawliczak-Maj*

The aim of the work was to determine the fur colour of Castorex rabbits. The studies were performed on 125 raw skins, using colorimeter, type Minolta CR-200. The colour was determined by its photometric brightness Y% and coordinates x and y. It was found that in respect of Y% indicators, i.e. x and y, the colour of the coat was similar in the middle of the back and on the butt as well as on the neck and the side. The attention was paid to tendency of colour's darkening in males.

### **Female chinchilla reproduction characteristics in their first breeding season**

*D. Dzierzanowska-Goryn*

Chinchilla breeding is constantly a very promising production. With expectation for substantial profits, producer's number grows every year despite of high investment costs. It should be remembered that a good profit in animal production comes out of good management, nutrition and above all, appropriate breeding material. This paper characterizes reproduction in new brand chinchilla farm. In spite of unexperienced personnel and medium quality of animals, the satisfactory results of reproduction, not differing from the mean values obtained in the recognized national farms, have been obtained.

### **Effect of probiotic preparation on the growth and the chosen parameters of hair cover quality of polar foxes**

*A. Gugolek, M.O. Lorek, W. Zablocki, A. Hartman*

The research material consisted of 80 polar Blue foxes, divided into 2 equal groups, with the same number of males and females. The studies were conducted during the period from weaning of puppies till the end of their growth and development of winter hair cover. The probiotic preparation, containing living spores of *Bacillus cereus* var. *toyoi* was administrated to animals of group II. It constituted the experimental factor. During the experiment, body weight measurements, as conducted in 2-week intervals, were analysed. Also, the results of body weight conformation were evaluated and analysed. The quality of skins was subjected to evaluation according to dimensions and category of hair cover fur and internal side of skin. Based on the obtained results of the studies, it was found that the addition of preparation with the living spores of *Bacillus cereus* var. *toyoi* to the feed did not have an influence on the increase of final body weight of polar foxes. On the other hand, it caused the improvement of parameters of hair cover quality, determined in living animal as well as post-mortem.

### **The results of the sale of Blue polar fox skins (the Finnish type) in season 2001/2002**

*S. Kubacki, R. Horoszczuk, P. Kubacki*

The carried out analysis of Blue polar fox skin sales (the Finnish type) in season 2001/ 2002, excluding the September auction (11-12.09.2002), showed a slight decrease by about 15% of mean skin price (in euro) in comparison to season 2000/2001. The decrease of mean price of sold skins in Helsinki concerned both Finnish and Polish skins (the Finnish type) reaching the average price on the level of 94,89 euro and 66,94 euro, respectively. Significant disproportion occurred between the mean auction price of skins and Polish skins (about 42%)

### **Macroscopic and microscopic evaluation of the freshly obtained semen of breeding raccoon dogs**

*P. Niedbala, O. Szeleszczuk*

The aim of the study was to perform preliminary and detailed evaluation of the semen of male raccoon dogs, obtained by the method of massage. The volume of the semen from breeding raccoon dogs varied from 0.1 to 1.5 ml, with the mean of 0.65 ml. Most of the ejaculates (more than 65%) was characterized by a white colour and 86% possessed a specific, weakly sensible odour, characteristic of this animal species. In the semen, milky and milky-watery consistency was dominating (in 63%); the best ejaculates with the cream consistency amounted to 16.44%. From among the ejaculates, thick and very thick densities were dominating. In the discussed ejaculates, more than 53% in average, and maximum, 90% of motile spermatozoa were found. From this quantity, 41-60% of spermatozoa revealed the progressive motility. In 1 ml of semen from  $145 \times 10^6$  and even to  $1305 \times 10^6$  of spermatozoa were found, with the average of  $478 \times 10^6$  of sperms.

### **Impact of air pollution on some haematologic indices in blood of polar fox (*Alopex lagopus* L.)**

*B. Nowakowicz-Debek, L. Saba, Z. Gradzki, A. Zietek, H. Bis-Wencel*

The investigations were designed to determine the influence of air contamination on some blood haematologic indices of Blue foxes. The animals were divided into two groups: the control one managed in farm conditions and the experimental group placed in the chamber with limited air movement, i.e. exposed to released pollutants. In the experimental group there were recorded lower values for RBC, HCT, PLT and higher for WBC as compared to the control. The picture of erythrocytic indices of the foxes from the experimental group, especially MCH and MCV indicated anaemia which is likely to appear due to the experimental factor activity.

### **Odour emission at the rabbit farm**

*B. Nowakowicz-Debek, L. Saba, L. Gacek, A. Lasek, O. Ondrasovic*

The studies conducted at the rabbit farm aimed at determination of sulphur compounds' emission level. The samples were collected in the breeding section (the basic herd section) and in the fattening section. There were altogether 12 sulphur compounds identified in this group. In the breeding section a high concentration was recorded for ethyl mercaptan ( $107,46 \text{ ug/m}^3$ ), while at the fattening sector-for methyl mercaptan ( $125,65 \text{ ug/m}^3$ ). For most of the identified compounds (except hydrogen sulphide) there are no standards regulating their level in the livestock buildings.

### **The effect of DL-methionine supplementation to the feed ration on body conformation and pelt quality of the common foxes**

*P. Przysiecki, S. Nowicki, Z. Nawrocki, F. Pawlak*

The aim of this study was to test the effect of DL-methionine on selected production traits in the common foxes. The experiment was carried out on 98 Silver common foxes in the period from birth to slaughter. The preparation was added to the standard feed ration. Results of this study indicate a positive effect of the DL-methionine on the investigated traits.

### **Effect of certain zootechnical procedures on production performance of Red common fox**

*P. Przysiecki, S. Nowicki, F. Pawlak, Z. Nawrocki, D. Stanislawski*

The aim of the work was to study the effect of catching and weighing of foxes on their production value. The studies were performed in the period from 20 May to the 10 December on the breeding farm of foxes P17, belonging to Agricultural

Cooperative Enterprise in Lubnica (the Wielkopolskie voivodship). They included 36 Red common foxes of both genders; the animals were divided into 2 groups according to their catching and weighing. In the control group, it was carried out at the beginning and the end of the experiment, in the experimental group once a month. In November, the conformation of foxes was evaluated and all of them were slaughtered and on their dry pelts, the length and weight and height of hair and undercoat, were determined. The category of hair and category of defects was determined and class of pelts was established. The repeated catching of Red common fox affected, in general, unfavourably the utility traits of the studied animals.

#### **The functional traits' level of mink with regard to both their ages and times of their mating**

*Iwona Rozempolska-Rucinska, Grazyna Jezewska*

The purpose of the studies was to evaluate the functional traits level on a fur-animal breeding farm of minks as well as to analyze the factors determining the level of the traits. The material for the study was represented by data obtained from breeding documentation of a fur-animal breeding farm of standard minks. Investigations covered 12 generations of animals. Reproduction evaluation was carried on 12 455 litters, which were born by 7376 brood females. Influence of the factors determining the level of studied traits was studied by many-factor analysis of variance by the Least Square Method, using procedure of a statistical package SAS (1996). The functional traits' level was expressed as a probability of appearance of a specific event depending on chosen factors (LSM) as well as standard error (Se,) describing the credibility of estimates. Among the brood females in the examined population of minks, there were 76.89% delivered and raised litters, 9.53% delivered and destroyed litters, 13.33% failed to deliver litters, however 0.25% were miscarriage litters. The age of females as well as the times of heating were the factors, which exerted an influence on the functional traits' level. The most favourable level of the functional traits was found among two-

years old females. In that group, there was the highest probability of delivered and raised litters (0.815) and little possibility of destroyed litters (0.075) as well as infertile females (0.106). Improvement of the functional traits' level could be obtained by the repeated mating of females. In case of the second-time service the probability of appearance of delivered and raised litters females in the population was increased from 0.682 (one time service) to 0.771 (three times mating). Simultaneously, the participation of infertile females was decreasing.

#### **The impact of the term of birth and the age of females on the reproduction results of the polar fox (*Alopex lagopus L.*)**

*S. Socha, D. Kolodziejczyk, A. Gontarz, E. Tomczuk*

The aim of the research was to analyse the influence of the kitting term and the age of females on the results of reproduction of polar foxes. The research was carried out on the breeding farm situated in the middle part of Poland. The present work is continuation of the previous research on this in subject Department of Breeding Methods and Fur Animal Production. The analysis concerned two basic traits connected with the reproduction: the number of born and weaned puppies in a litter per vixen. The analysis of variation for the number of born puppies showed statistical influence of the age of females bred on the farm. In relation to the number of reared puppies it has been proved that the age of females and the calendar year was statistically significant. However statistical significance of the birth term had not been visible in relation to the analyzed traits. The mean number of born puppies and weaned puppies was lower in the herd of one year old vixens and six years old. The highest values of the traits were obtained in the group of three years old vixens. The obtained results suggest a slight impact of the birth term on the polar fox vixens' reproduction results. Litters in the early births were more numerous in comparison to the later kittenings of females. The obtained results of the reproduction seem to be very good.

### **Cases of dental diseases in breeding chinchillas (*Chinchilla laniger M.*)**

*M. Sulik, B. Seremak, Z. Muszczyński, M. Wachowiak*

In connection with a fact of more and more frequent cases of dental defects in chinchillas, amounting to ca 20% in the analysed herd, the attempts have been undertaken with the aim to

examine the reasons for this situation, and to establish the types of occurring defects. The studies were conducted in a farm, situated in the Western Pomerania region and included females, culled from the herd, with the symptoms characteristics of dental defects. In the paper, the particular cases of hypertrophy of incisors, pre-molars and molars were described. It has been found that the problems of dental defects become more and more serious breeding problem. The discussed disease is incurable and leads to unavoidable death of animal. The method of early diagnosis of dental defects in farm management includes palpal examination of mandible in order to feel the hypertrophy of dental roots. Such procedure may abbreviate suffering of animals and allow the breeder to obtain a skin before it is destroyed by the disease.

### **Acceleration of oestrus period in female polar and common foxes, with the maintenance of light requirements**

*O. Szeleszczuk, S. Jarosz*

Period of oestrus incidence and by this, the date of the female's service affects significantly the reproduction results and profitability of breeding of polar and common foxes. The sexual cycle of foxes is closely related to the length of the light day. The decreasing length of the day commences all hormonal changes in animal body which, in turn,

affect the functional state of gonads and appearance of reproduction organs of females as well as males. In the work, there were presented the results of the studies which showed the effect of changes in the length of the light day on acceleration of oestrus or the change of the period of its occurrence, and on spermatogenesis.

### **The existing tendencies in fox and mink breeding in Bydgoszcz Breeding District in the period 1992-2002**

*J. Zawislak1, B. Laski, S. Kubacki*

The aim of the analysis was to get familiarized with the existing tendencies in polar fox, common fox and mink breeding on the farms in Bydgoszcz Breeding District in the period 1992-2002. On the basis of the carried out analysis it has been shown that the investigated animal species had decreasing tendencies in the number of females in the basic stock. It has been ascertained that polar fox and common fox vixens had high fertility rate (98.88% and 97.47% respectively) and minks had the highest rate of reared pups (90.69%). Taking into account the number of born and reared pups per litter in the fox herd we can perceive increasing tendency but a slight decreasing tendency appeared in the stock of minks.

## Faglig Årsberetning

2004

Pelsdyrerhvervets Forsøgs- og ForskningsCenter



## Annual Report

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Danish Fur Breeders' Research Center  
 Herningvej 112 C  
 DK-7500 Holstebro  
 Phone: +45 96 13 57 00  
 Fax: +45 97 43 52 77  
 E-mail: [pfc@kopenhagenfur.com](mailto:pfc@kopenhagenfur.com)

### Reports on: Behaviour

#### Behaviour around birth in relation to early kit mortality in mink

*M. Gade, J. Malmkvist*

Early kit mortality is of a considerable size in mink production, and a reduction in the mortality would

be an advantage. Based solely on kit counts, the mortality rates between birth and weaning average 20-30%; however, direct observations of births indicate that the real numbers are higher – at least as regards the one-year-old females examined. Based on direct observations of pregnant female mink before, during and after birth as well as collection and autopsy of all the dead kits, in 2004 the purpose of the project was to study a number of factors of

importance in relation to early kit mortality. In the study 84% of all the dead kits died within the first 24 hours after birth, and the percentage of stillborn kits was 42%. The duration of the births differed between the females (mean  $\pm$  sd.: 9 h 3 min  $\pm$  6 h 8 min), and the duration had an effect on the number of kits that died within seven days after birth. The behaviour of the females before, during and after birth was analysed systematically, and the litters of females with high kit mortality rates were often left lying around compared to the litters of females with low kit mortality rates. The study demonstrated that the duration of birth as well as some, but not all the behavioural patterns of the female mink had an effect on early kit mortality. This knowledge is of importance in relation to future studies aiming to reduce the relatively high early kit mortality in mink.

*Annual Report 2004, 7-14. Danish Fur Breeders' Research Center, Holstebro, Denmark.*

### **Fibres in mink feed in the winter period. Influence on behaviour and reproduction**

*T.N. Clausen, S.W. Hansen, B. Houbak, C. Hejlesen*

In the winter period we used 3 groups, each consisting of 178 scanblack mink females, to investigate the effect of citrus pectin offal and the amino acid tryptophan on the time without feed, the stereotypic behaviour of the females and their reproductive results. A control group (KON) was fed a typical grower feed (energy distribution, protein:fat:carbohydrates; 30:52:18). To one group we added 10 percent citrus pectin offal (PEC) and to one group we added tryptophan up to a total of 0,30 g digestible Trp / 100 kcal. Trial feed was fed from Dec. 19 to Feb. 24. After that, feed from the local feedkitchen was fed to all groups.

It was concluded, that citrus pectin offal and tryptophan in January had no influence on the female's time without feed and her stereotypic behaviour, probably because the feed reduction was too high. In February we found that citrus pectin offal in the feed and to a lesser extent tryptofan

reduced the time without feed. However the reduced time without feed did not reduce stereotypic behaviour, so the taste of these products is probably more important for the reduction in time without feed than satiety.

The products had no negative effect on the reproduction results, and they could not reduce stereotypic behaviour.

Females eating fast in January and February are those females that loose much weight in the same period. These females also are low in weight, eat fast and have stereotypic behaviour.

*Annual Report 2004, 15-26. Danish Fur Breeders' Research Center, Holstebro, Denmark.*

### **Effect of cage enrichment on the welfare and productivity of mink**

*S.W. Hansen, J. Malmkvist*

The effect of cage enrichment was studied in 129 pairs of mink kits born and reared in standard or enriched cage environments. During rearing the mink had access to one or two cages. The enrichment of the cages consisted of tubes made of either wire mesh or plastic and thin nylon ropes. The results demonstrated that enrichment of mink cages improves the welfare of the animals in that less tail chewing, fewer social interactions and a reduced level of cortisol (stress hormone) are observed. Cage enrichment does not affect the feed consumption or the body weight, but it reduces the consumption of straw. Access to one or two cages has no effect on the behaviour of mink, tail biting, feed consumption, body weight, cortisol level, or the consumption of straw or nylon ropes. Thus, in standard cages increased complexity in combination with occupational materials may improve the welfare of mink whereas a doubling of the size of mink cages has no effect in relation to improved welfare.

*Annual Report 2004, 27-38. Danish Fur Breeders' Research Center, Holstebro, Denmark.*



### **Tow steps forward and three steps back – group housing of mink**

*S.W. Hansen, B. Houbak*

On a Danish mink farm the incidence of fur chewing and biting marks was compared in mink kept in pairs in standard cages as well as in groups in stacked cages. The study included an examination of how the composition by sex and family relations affect the incidence of fur chewing and biting marks in mink kept in stacked cages. 164 pelts from mink kept in standard cages and 634 pelts from mink kept in stacked cages were examined. The social stimulation in the groups or the complex environment of the stacked cages reduced the incidence of fur chewing compared to the mink kept in standard cages. The mink kept in standard cages had significantly fewer bite marks than the mink kept in stacked cages. Also, the female mink had fewer bite marks when kept with a male in a standard cages compared to when kept in groups of female mink. Whether the mink were kept in standard or stacked cages did not make any difference as regards the extent of the biting marks; however a significant correlation was found between the extent of neck, back, hip and tail head bite marks. Compared to the males, the female mink were more exposed to bite injuries when kept in groups. On the basis of the increased incidence of bite marks it is concluded that the welfare of mink is reduced when the animals are kept in groups of males and females or in groups of only females.

*Annual Report 2004, 39-47, Danish Fur Breeders' Research Center, Holstebro, Denmark.*

### **Reports on: Breeding and reproduction**

#### **Mapping of the mink genome**

*K. Christensen, R. Anistorael, A. Farid*

Genetic and physical maps have been used for the identification of genes that modulate monogenic traits, or for the identification of chromosomal regions which contain genes having a major effect on economically important traits (QTL's). Despite the economic importance of mink production in Northern Europe and North America, mink genomics research is lagging far behind other

livestock species. The objective of our work is to create the first generation of linkage map of the mink genome with a resolution of at least 20 cM. A 200-300 markers would be needed to reach this resolution. This map will serve as a basis for further refinement. Genotypes of a reference population consisting of four males, nine females and 72 F1 progeny were typed at 65 informative polymorphic markers (microsatellite loci). These markers were assigned to several linkage groups using Crimap software; the largest linkage group contained 4 markers. Physical mapping of the markers was also performed using a panel of mink-hamster hybrid somatic cell lines, showing consistent results with the linkage map. The markers can be used for paternity testing in the mink. A complete map is expected to be produced within the next year, it will be possible by means of the map and homology between species to retrieve information from the extensive map for man and mouse. And one of the first application of the map will be identification of the biological function of the colour genes segregating in the mink.

*Annual Report 2004, 49-53. Danish Fur Breeders' Research Center, Holstebro, Denmark.*

#### **Mink selected to produce on a low protein content in the feed. Status for growing period 2003 and lactation period 2004**

*T.N. Clausen, C. Hejlesen, P. Sandbøl*

After pelting 2002 we started an investigation on the possibility of breeding mink with a good fur quality when the content of protein in the feed was low, without negative consequences for reproduction and growth.

In the growing furring period 2003 the pelt length was lower in the investigation group than in the control group, but for both groups the skin length had increased from 2002 to 2003. There was no difference in fur quality.

In the breeding period 2004 the control group gave birth to and weaned more kits than the investigation group. The weight of the kits at weaning was also higher in the control group than in the investigation group. Female body weight differed between groups throughout the winter period.

*Annual Report 2004, 55-59. Danish Fur Breeders' Research Center, Holstebro, Denmark.*

### **Reports on: Nutrition and feeding**

#### **Vitamin E to mink females and its transfer to kits**

*S.K. Jensen, M.U. Hansen, T.N. Clausen*

Vitamin E ( $\alpha$ -tocopherol) is a very important antioxidant for minks and beyond its function as an antioxidant in cell membranes vitamin E is very important for the development and maintenance of the immune system. Vitamin E is added to the feed as synthetic all-rac- $\alpha$ -tocopheryl acetate. The synthetic form consist of an equal amount of 8 stereoisomers of  $\alpha$ -tocopherol, while  $\alpha$ -tocopherol synthesized by nature always posses the RRR configuration. Several investigations have shown a higher utilization of the natural form of vitamin E compared to the synthetic form, caused by a biodiscrimination within the animals. In an experiment with lactating mink the transfer of the different isomers of  $\alpha$ -tocopherol from feed to mink, mink milk and further to the mink kits was studied. Fifteen Scanbrown female mink was included in the experiment, they were fed with traditional farm feed containing 60 mg all-rac- $\alpha$ -tocopheryl acetate per kg feed. Thus the natural isomer constituted only 16% of the  $\alpha$ -tocopherol in the feed Blood samples were taken in April before birth and 28 and 42 days after birth. Milk samples were taken at day 2 and 28 after birth. From the kits plasma and tissue samples was taken 28 days after birth. Analysis of the vitamin E content and of the stereochemic composition of  $\alpha$ -tocopherol showed that the natural isomer were the dominating isomer in plasma and milk from the mothers as well as in plasma and tissues from the kits. In heart muscle the natural isomer made up 60% of the total  $\alpha$ -tocopherol content. Based on the relative abundance of the different stereoisomers it can be calculated that mink utilize RRR- $\alpha$ -tocopherol a factor 2.5 – 3 times better than all-rac- $\alpha$ -tocopherol.

*Annual Report 2004, 61-67. Danish Fur Breeders' Research Center, Holstebro, Denmark.*

#### **Fat soluble vitamins and antioxidants in mink feed tocopherols and carotenoids in oilseed rape and lupnes**

*M.R. Clausen, S.K. Jensen, J.C. Sørensen, H. Sørensen*

Tocopherols are important constituents in feed, partly because of their protective action against lipid oxidation, and partly because of their vitamin E effect. Among the eight naturally occurring tocopherols exists differences in relative antioxidative effect as well as in their effect as vitamins. In order to evaluate the quality of different feed sources and feeds, effective methods for analysis of these components are necessary. Carotenoids act as antioxidants as well. However, since the mechanism of action of carotenoids is fundamentally different from that of tocopherols, the content of carotenoids should be evaluated as well. Methods of analysis are developed for determination of individual tocopherols and carotenoid and the results from analysis of two potential lipid and protein sources for mink feed, lupine and rapeseed, are presented. The results show, that especially lipids in lupine are effectively protected against oxidation, partly because of a beneficial composition of the tocopherols, partly because of a high content of antioxidants. The high content of polyunsaturated fatty acids in mink feed, makes it extremely exposed to lipid oxidation, and thereby reduction of quality. Therefore, it is necessary to add antioxidants, and sources of natural tocopherols are therefore of great interest. A potential source is the deodorizer distillate (DOD) from plant oil production, which is a relatively cheap by-product available from commercial oil mills with tocopherol concentrations of 1 - 2 %. Enrichment is necessary in order to make this source of tocopherols more attractive for food and feed applications, and here preliminary experiments with two techniques are presented: short-path distillation and supercritical CO<sub>2</sub> (SC-CO<sub>2</sub>) extraction.

*Annual Report 2004, 69-75. Danish Fur Breeders' Research Center, Holstebro, Denmark.*

### **Protein to mink in the nursing period and in the period of early kit growth**

*T.N. Clausen, C. Hejlesen, P. Sandbøl*

To investigation on the need for protein in the winter and lactation periods, we used 7 groups of each 128 scanbrown mink females. A control group (K50) was feed 50 % of metabolisable energy (OE) from protein in the whole investigation period. The other groups were feed typical Danish growth period feed, 30 % of OE from protein, until February 24. Group K30\_45 continued on growth period feed until April 20, thereafter the females were feed 45 % of OE from protein in the rest of the investigation period. Group K45 was feed 45 % of OE from protein from February 24. The remaining four groups were feed 45 % of OE from protein from February 24 until April 20; thereafter they had 30 % of OE from protein until May 28. Group D30 continued on 30 % of OE from protein until weaning. Group D45 changed to 45 % of OE from protein until weaning. Group D\_A/L had 45 % of OE from protein with an arg/lys content on 1.15 in the feed, and the last group (D\_M) was fed a feed mixture optimised to an amino acid profile resembling the content in mink milk.

The results showed that mink kits who in the nursing period or from day 28 until day 42 in the nursing period were feed 45 % of OE from protein had the best kit weights day 42. 30 % of OE from protein to the kits from 28 days of age is too little protein and changing the amino acid profile to resemble the profile in mink milk had no positive effect on kit growth, neither had a changed arg/lys content in the feed.

*Annual Report 2004, 77-81. Danish Fur Breeders' Research Center, Holstebro, Denmark.*

### **Effect of protein, fat and carbohydrate supply on growth and energy metabolism in mink kits**

*R. Fink, A.-H. Tauson, N.E. Hansen*

Growth rate of mink kits from parturition until 9 weeks of age, and energy metabolism after weaning (7 weeks post partum) were measured by means of weekly weight recording, and balance and respiration experiments during weeks 8 and 9 post

partum. The kits were raised under conventional farm conditions by dams fed ad libitum from parturition with one of 3 diets containing different energy ratios (% of metabolisable energy (ME)) between protein:fat:carbohydrate: high protein (HP): 60:35:5, medium protein (MP): 45:40:15 or low protein (LP): 30:45:25. Live weights 4 weeks post partum were highest ( $P<0.05$ ) in kits nursed by dams fed the LP diet, whereas live weights 9 weeks post partum were highest ( $P<0.05$ ) among kits fed the MP diet. During weeks 8 and 9, ME intake was not affected by dietary treatment. However, nitrogen (N) intake, total water intake and N excretion were highest ( $P<0.05$ ) in kits fed the HP diet, whereas the N balance was highest ( $P<0.05$ ) in kits fed the MP diet. Heat production (HE) was not affected ( $P>0.05$ ) by dietary treatment, but retained energy (RE) and protein oxidation (OX) was higher ( $P<0.05$ ) whereas oxidation of fat and carbohydrates were lower ( $P<0.05$ ) in kits fed the HP diet than in kits fed the LP diet. In conclusions, live weights of the kits 4 weeks post partum indicated that dams fed the LP diet had the highest milk yield. However, during transition from milk to solid feed and after weaning, kits fed the MP diet had the highest growth rate, which may be caused either by the kits' lower ability to utilise the high carbohydrate content of the LP diet or, that they have a higher protein requirement. However, further experiments are needed to elucidate the actual nutrient requirement of mink kits during transition from milk to solid feed and at weaning.

*Annual Report 2004, 83-87. Danish Fur Breeders' Research Center, Holstebro, Denmark.*

### **The amino acid requirements of mink (*Mustela vison*) in the intensive growing period – growth results**

*T.B. Nielsen, P. Sandbøl, C. Hejlesen, N.E. Hansen, J. Elnif*

90 Scanbrown male mink kits were divided into two groups, early (24th – 25th April) and late (3rd – 4th May) born, each group consisting of 45 kits and these were again divided into feeding groups consisting of 5 kits. Both kit groups were used in two experimental periods, and the kits were between 58 – 59 days old when the first experimental period was initiated, and between 75 and 76 days old when

the second experimental period was initiated. Between the two experimental periods the kits were kept separately in standard cages (with nesting boxes) on the farm. During the experimental period (8 full days causing 9 calendar days) the kits were kept separately in cages designed for Nitrogen Balance Trials having separate urine and faeces collection, with free access to water (drinking nipples) and a feeding channel where the feeding bowls were placed. The experiment was carried out from the 21st of June until the 26th of July 2004 which is a period of intensive growth for the kits.

An estimated ideal amino acid profile was investigated by increasing or decreasing a single essential amino acid (EAA) by 20 % in order to determine the kits actual need for EAA in this period of intensive growth. The 12 EAA, and semi EAA, investigated in this experiment was: arginine, cystine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine, tryptofane, tyrosine and valine. All the feed mixes contained 18,3% of ME from raw protein and the composition of the raw materials were typical feedstuffs with a high digestibility. Synthetic EAAs were added to obtain the different levels of each EAA.

Only for valine was a significant difference ( $p < 0,0426$ ) found in the growth rate (weight gain/initial weight (%)) from the estimated ideal level, which was observed when the level was deleted by 20%. There was no effect on the growth rate by adding 20% of the estimated ideal level for valin. This indicates that the estimated ideal level is correct. Lysine and threonine did show significant differences in growth rate in the 4-day adjusting period, but these were both tested in the first experimental period run in the experiment, and in this period the kits were not fed ad libitum as planned. Because the kits were not fed ad libitum in the first experimental period, the growth rate displayed a big dependency on the amount of feed given, and therefore the results are inconclusive. For the remaining EAAs there was no significant difference between the levels, a fact that could suggest that the level estimated as the ideal level could be lowered. There was a significant difference ( $p < 0,0001$ ) in the collection period between the growth rates in the first experimental period (61 – 66 day old) and second experimental period (78 – 83 day old) for both the early and late born kits.

*Annual Report 2004, 89-102. Danish Fur Breeders' Research Center, Holstebro, Denmark.*

### **Sulphur containing amino acids for mink in the period of growth**

*P. Sandbøl, T.N. Clausen*

Present norms for mink state the need of digestible methionin (met) as 0.16g/100 kcal metabolisable energy (ME) and cystin (cys) as 0.06 g/100 kcal. In this way met makes up 73% of the total norm for sulphur-containing amino acids (SAA). For cats the norm is stated as 0.11 and 0.11g/100 kcal and so met makes up 50% of SAA.

Trials have indicated that mink cannot make use of d-met; whereas cats for some extent can make use of it and cats can also for some extent make use of a methyl hydroxy analog (MHA) instead of met.

Two trial series were carried out, partly to examine the effect of changing the met/cys proportion and partly to examine the mink's ability of making use of d-met and MHA respectively.

In the first trial series the total content of SAA was 0.22 g/100 kcal and met made up 0.15; 0.14 and 0.12 g/100 kcal respectively. Group 0.16 had significantly the best gain for the whole period and group 0.12 had significantly the poorest. From the 25th of September till pelting there was no difference between the groups. However there was a tendency of more dead kits during this period with a decreasing met-content. Group 0.12 had significantly shorter pelts and poorer pelt quality than the two other groups. At pelting there were no differences in the relative liver weights (RLW) or the fat content of the liver.

In the second trial series we used a basic blend with 0.11 g digestible met/100 kcal. This blend was added dl-met or MHA, so that all blends contained a digestible/utilizable amount of met of 0.16 g/100 kcal.

The point was to make use of 100%, 50% or 0% of d-met, why the total met content of the blends became 0.16; 0.19; 0.22 and 0.10 (+MHA) g/100 kcal respectively.

Group 100% and group 50% had a significantly larger gain from July till the 4th of September than group 0% and group MHA. For the rest of the sub-periods and for the period of growth as a whole, there were no significant differences between the groups. There were no statistically significant differences between the groups in any of the skin-parameters. There were no differences in the mortality of the groups. At pelting there were no differences in the relative liver weights or the fat contents of the livers.

For the first series it can be concluded that with a total content of SAA of 0.22 g digestible/100 kcal it seems that the ratio of methionin to cystin of 0.16 g met / 0.06 g cys per 100 kcal is the optimum for mink.

For the second series it can be concluded that the d-form of methionin seems to be used or that 0.10 (natural content) + 0.02 (added l-met) g digestible methionin per 100 kcal is enough, or that large amounts of total methionin in the feed reduce the growth in the beginning of the period of growth.

In the furring period it has no influence whether the methionin is added as d/l-methionine or replaced by Alimet. The total need of sulphur containing amino acids might be reduced in the period from September till pelting compared to the present norm.

*Annual Report 2004, 103-109. Danish Fur Breeders' Research Center, Holstebro, Denmark.*

### **Ideal protein for mink (*Mustela vison*) in the growing and furring periods**

*P. Sandbøl, T.N. Clausen, C. Hejlesen*

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 autopsied. 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.

*Annual Report 2004, 111-119. Danish Fur Breeders' Research Center, Holstebro, Denmark.*

### **Cracklings to mink in the growing period. Influence on health, growth and fur quality**

*T.N. Clausen, C. Hejlesen*

Five groups each consisting of 120 scandblack male- and 120 female mink kits were fed increasing amounts of cracklings in the growing- furring period. The cracklings substituted particularly fish products and were used in 10, 20 and 30 percent wet

cracklings and 6.3 percent dry cracklings. Further there was a control group without cracklings.

The results showed that cracklings can be used in the growing- furring period in amounts of 30 percent, wet cracklings and 6.3 percent, dry cracklings, without negative effect on pelt length and skin quality. However it should be noted that the amount of phe + tyr should be at least 0,53 g / 100 kcal, to get a dark colour of the skins.

*Annual Report 2004, 121-124. Danish Fur Breeders' Research Center, Holstebro, Denmark.*

### **Acidified poultry offal to mink in the growing period. Influence on health, growth and fur quality**

*T.N. Clausen, C. Hejlesen*

Four groups each consisting of 120 scanblack male- and 120 female mink kits were fed increasing amounts of acidified poultry offal in the growing-furring period. The acidified poultry offal were used in 10, 20 and 30 percent, further there was a control group with 10 percent Danish poultry offal instead of acidified poultry offal.

The results show that acidified poultry offal can be used in the growing- furring period in amounts of 30 percent, without negative effect on pelt length and fur quality.

*Annual Report 2004, 125-127. Danish Fur Breeders' Research Center Holstebro, Denmark.*

### **The effect of protein level on N-balance in adult mink (*Mustela vison*)**

*C. Hejlesen*

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<sup>0.75</sup> 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 ( $\div 1.6 - \div 8.3$  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 (12%, 19% and 21%,  $p < 0.05$ ) as dietary ME from protein increased.

*Annual Report 2004, 129-135. Danish Fur Breeders' Research Center, Holstebro, Denmark.*

### **Water as a satiety factor in mink**

*C. Hejlesen, P. Sandbøl, K. Gregersen*

In a period without production, the energy requirement of a mink is low. The allotted quantity of energy is ingested within less than 24 hours. The time without feed is believed to be a releasing factor in the development of stereotypes, and is therefore undesirable.

It was investigated if lowering the energy concentration of the feed by addition of water could extend the time spent eating a limited quantity of energy. In addition it was investigated how much feed males, fed ad libitum, ate at different hours after feeding, in percent of total daily intake.

It is concluded that a reduction of the energy concentration in the feed by lowering the dry matter content (DM) (by addition of water), from 49% to 39% does extend the time mink spend eating a limited quantity of energy. A reduction to a DM of 32% does not extend the time any further.

It is also concluded that elimination of a time interval without feed intake is not likely, as the ad

libitum fed males ate almost 90% of their daily energy intake within 14 hours after feeding.

*Annual Report 2004, 137-142. Danish Fur Breeders' Research Center, Holstebro, Denmark.*

### **Content and *In Vivo* digestibilities in feed ingredients for mink feed**

*C. Hejlesen*

Danish Fur Breeders' Research Center (DFBRC) has performed 86 digestibility trials with feed ingredients used in the Danish production of mink feed. The contents of dry matter, crude ash, crude protein, crude fat and crude carbohydrate is presented along with the measured apparent digestibilities of these nutrients. For 65 of the 86 ingredients the content of amino acids and their measured apparent digestibilities is presented.

The results are to be used when updating the "Råvaretabel", which is used by the Danish central feed kitchens producing mink feed.

*Annual Report 2004, 143-150. Danish Fur Breeders' Research Center, Holstebro, Denmark.*

### **Reports on: Physiology and analytical techniques**

#### **Physico-chemical properties of carbohydrates and their effect on digestion in mink**

*H.N. Lærke, M.S. Hedemann, C. Hejlesen*

Studies in other monogastrics have shown that viscosity and water binding capacity (WBC) influence feed intake, rate of passage, enzymatic activity, and 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 (4 x 8) were offered 1.26 MJ metabolisable energy (ME) once daily for 4 days followed by 1.05 MJ ME for 6-8 days. 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. There was no effect of carbohydrate source on pancreatic enzyme activity, but differences were seen in the enzyme activity in intestinal mucosa. However, these differences were not correlated to the observed differences in total tract digestibility.

In all groups, there were large variations in the amount and rate of feed consumption with no effects of diet composition.

*Annual Report 2004, 151-159. Danish Fur Breeders' Research Center, Holstebro, Denmark.*

#### **Effects of pelting and feed on the distribution of gastrointestinal content in mink**

*H.N. Lærke, C. Hejlesen*

In order to perform studies on digestion processes in the gastrointestinal tract on a larger number of individuals, we conducted a study to test if pelting affected the distribution of gastrointestinal contents and an added marker. In the experiment 4 x 8 adult male mink were fed a control feed (2 x 8 males) or a feed with 10 % of added citrus fibre. After an 8 d. adaptation period the mink were killed 3 hours after feeding on d 9 or 10. Half of the males were pelted immediately after euthanasia, whereas the other half of the animals was not. The gastrointestinal tract was gently removed from the carcass, and the amounts of gastrointestinal contents and number of plastic pellets was analysed. Pelting affected the distribution of gut contents and plastic pellets. Measurements of transit time and other studies involving detailed sectioning of the gastrointestinal tract should thus not be performed on pelted animals. However, in studies where detailed sectioning is not in question, and where a large number of animals is necessary, pelting can be included if performed immediately after euthanasia.

There was a tendency for a reduced amount of contents and smaller number of pellets in the stomach at the expense of an increase in the distal gastrointestinal tract when the mink were fed the diet containing the citrus by-product. This indicates that the added of this bulk material increases the rate of passage through the gut.

*Annual Report 2004, 161-167. Danish Fur Breeders' Research Center, Holstebro, Denmark.*

### **Correlation between liver fat and dry matter in mink (*Mustela vison*)**

*T.N. Clausen, P. Sandbøl*

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 be 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 =  $1.15 * \text{liver dry matter} - 24.9$  ( $R^2 = 0.97$ ). It is concluded that this method can be used for a quick, cheap and acceptably precise evaluation of liver fat content.

*Annual Report 2004, 169-171. Danish Fur Breeders' Research Center, Holstebro, Denmark.*

### **Mink proteinases; trypsin and chymotrypsin and the effect of proteinase inhibitors**

*T.N. Clausen, M. Kjelgaard-Hansen, K. Mortensen, A.D. Sørensen, J.C. Sørensen, H. Sørensen, S. Sørensen*

Protein utilisation in mink and other monogastric animals highly depend on protein digestion/hydrolysis catalysed by proteinases in the stomach and in the proximal part of the small intestine. Especially trypsin (EC 3.4.21.4) and to some extent also chymotrypsin (EC 3.4.21.1), which are produced from the pancreatic proenzymes trypsinogen and chymotrypsinogen, respectively, have key functions in protein hydrolysis. The activity of proteinases can, however, be blocked or inhibited by a group of different proteins (proteinase inhibitors) present in the diet. These inhibitors bind irreversible to the active sites of the enzymes. The result is excretion of undigested enzyme and inhibitor in a complex, which reduce the utility of the protein present in the diet, and it gives thus decreased absorption of amino acids. Proteinase inhibitors occur in varying amounts in different foods and feeds. This is the case for vegetable foodstuff, cereals, rapeseed, potatoes, and legumes, where especially soybeans and soybean products have high amounts of such inhibitors in native and insufficiently heat treated materials. Relatively high amounts of trypsin inhibitors are also found in potatoes and egg white proteins. The various proteinase inhibitors from the different sources are structurally very different. However, they all share the capability to bind strongly to the active sites of the enzymes resulting in lost enzyme activity. The present study shows that proteinases and proteinase inhibitors binds to each other in the ratio 1:1 (equimolar ratio) and the animals excrete thereby 1 mole of enzyme for every mole of undenaturated proteinase inhibitor present in the diet. Knowledge about proteinase inhibitors are, thus, of particular



concern in relation to digestibility of proteins in mink. The inhibitors give also reasons to concerns about the consequences of proteinase inhibitors on the biosynthesis of pancreatic proenzymes. It is found that a given amount of trypsin inhibitor has approximately ten times higher effect on mink trypsin than on porcine trypsin. Together with informations of binding ratios between enzyme and inhibitor, such data show that the mink trypsin has approximately ten times higher proteolytic activity than porcine trypsin. This is in accordance with the relatively short digestive tract in mink and the high transit times for the feed in mink. For mink kits these issues are even more pronounced and problems related to feed quality will become especially obvious. Evaluating protein digestibility based on in vitro methods, the type of proteinase used have also to be considered. Proteinases from mink and especially mink trypsin have been shown to be both structural and functional different from that of other animals. Two different methods have been established to isolate and characterise trypsin. Essential for both methods are the isolation of inactive trypsin and thus prevention of self hydrolysis of trypsin. The first method is based on separation of trypsin in complex with trypsin inhibitor (KSTI from soybean). The second method for isolation of trypsin utilise the knowledge of chemical properties of trypsin activity. The performed study show markedly differences in physical and biochemical properties between mink trypsin and porcine trypsin. Mink trypsin has higher activity (10 times higher) than observed with porcine trypsin and at least 2 isoforms of mink trypsin with molecular weights of 22-30 kD have been isolated: one with low pHi approximately 5.3 and the other with pHi > 11.

*Annual Report 2004, 173-184. Danish Fur Breeders' Research Center, Holstebro, Denmark.*

### **Lipids and metabolic processes in mink brains**

*T.N. Clausen, K. Mortensen, A.D. Sørensen, J.S. Sørensen, H. Sørensen*

Lipids in the brain of mink kits (*Mustella vison* Schreb) have been investigated with focus on the

special significance of lipids for brain structure and metabolism. Lipid analyses of mink feed and brain tissues obtained from mink kits have been performed by gas chromatography (GLC) to characterise the profile of fatty acids (FA). Supercritical Fluid Techniques (SFT; extraction, SFE and chromatography, SFC) have been used for the studies of intact lipids in feed and brain tissue. This study revealed that no intact triacylglycerols (TAG) are present in brain tissues of mink kits. However, FA is present in complex lipids e.g. phospholipids and glycolipids, accounting for 38 - 42 % of the brains content of dry matter (DM), which also is dominated by protein (47 - 50 % of DM). The brain lipids seems, thus, to consist primarily of membrane lipids with special significance for brain structure and function and they seem to play a minor role in metabolic processes for energy production. The brains energy is mainly produced from glucose and under special circumstances also from ketone bodies. The lipid metabolisms of the brain include enzyme catalysed processes as FA chain elongation (elongases) and formation of double bonds in FA (desaturases). The FA transported to or formed in the brain result, thus, in the mandatory long chain polyunsaturated fatty acids (LCPUFA). In this connection, the content of unsaturated FA in the feed seems to be significant as is a correct ratio between n-6 and n-3 PUFA and therefore also the formation of 20:4 (n-6) (AA; arachidonic acid), 20:5 (n-3) (EPA; eicosapentaenoic acid) and 22:6 (n-3) (DHA; docosahexaenoic acid). These LCPUFA's are regarded as essential for normal development of the brain, of visual function, and for optimal development of new born kits. An other special and very important information obtained with the present study, is the detection of quantitative dominating amounts of cholesterol in the brain tissue. Thus, knowing that the blood-brain barrier prevent transport of cholesterol from blood to brain, this strongly indicates that the dominating biosynthesis of cholesterol in the brain takes place with an assumed origin in ketone bodies, which are transferred from blood to brain.

*Annual Report 2004, 183-192. Danish Fur Breeders' Research Center, Holstebro, Denmark.*

### **Collecting and storage of urine in N-balance trials with mink**

*S. Lisbjerg*

In N-balance trial with mink urine is collected in bottles added sulphuric acid to prevent evaporation of volatile N. Further more the voided urine is collected daily and stored frozen until analysis. Experiments were conducted to evaluate if daily collection and storage at frost is necessary. N and pH in urine were measured as an effect of storage temperature and –time and addition of sulphuric acid.

The results showed that the content of N in urine collected in metabolic cages was unaffected by temperature and addition of sulphuric acid for 3-4 days. Without sulphuric acid the urinary pH increased from 7.2 after one day to 9.5 after 3 days. It is concluded that performing N-balance trial with mink and a 4 days long collections period does not require a daily collection of urine. It is however recommended to add an adequate quantity of sulphuric acid in the urine collection bottles.

*Annual Report 2004, 193-195. Danish Fur Breeders' Research Center, Holstebro, Denmark.*

### **Reports on: Health**

#### **Detection of mink astrovirus (MiAstV) in clinical samples with real-time RT-PCR**

*K. Ullman, K.-O. Hedlund, A.S. Hammer, M. Isaksson, H.-H. Dietz, L. Englund, G. Czifra*

A novel real-time polymerase chain reaction assay (real-time PCR) has been developed and evaluated for the detection of mink astrovirus (MiAstV) in clinical samples. Faecal samples collected from 218 mink kits during 1999 and 2003 from Danish and Swedish mink farms were analysed with both PCR and electron microscopy (EM). There was a very good correlation between the two methods. Astrovirus was detected with both EM and PCR in 75 samples while it was not detected with either methods in 106 samples. In 37 additional samples astrovirus could not be detected with EM, but they were positive when tested with PCR. The real-time PCR proved to be a more sensitive method than the

EM, it can be automated and is suitable for analysis of large numbers of samples. All these advantages make the PCR a useful diagnostic tool for large-scale routine detection of mink astrovirus.

*Annual report 2004, 197-201. Danish Fur Breeders' Research Center, Holstebro, Denmark.*

#### **Cloning and expression of the capsid protein of mink astrovirus**

*C. Baule, K. Ullman, U. Hellman, H.-H. Dietz, L. Englund, G. Czifra*

Genetic information available from earlier studies (Mittelholzer et al. 2003a) was used to produce a PCR product containing the MiAstV capsid protein gene. Targeted insertion of the gene into cloning and expression vectors of the Gateway series resulted in one recombinant protein expressed in bacterial cells. Expression of the same protein in mink lung and kidney cells was not successful. When the approximately 85 kDa protein was subjected to peptide mass fingerprinting, the analysed sequences were identical to peptides of MiAstV capsid protein. Results of preliminary studies with sera raised to potentially antigenic sites of the capsid protein also suggest that the expressed protein is identical to the capsid protein of MiAstV.

*Annual Report 2004, 203-208. Danish Fur Breeders' Research Center, Holstebro, Denmark.*

#### **Greasy kits**

*T.N. Clausen, H.-H. Dietz*

“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.

*Annual Report 2004, 209-214. Danish Fur Breeders' Research Center, Holstebro, Denmark.*

### **Investigation of urine pH and diseases in the bladder in mink kits in June and July**

*V. Weiss, T.N. Clausen, M.U. Hansen, A.M. Henriksen*

In the period June 9 to July 9, the incidence of bladder infections in mink kits was investigated. To the investigation we used 9 farms who received feed from 3 different feed kitchens. Three times during the investigation period we examined urine samples from 3 x 15 male mink kits (15 black, 15 brown and 15 light colour type) on each farm. Urine pH was examined, immediately thereafter the urine was frozen until microscopic examination. Dead mink kits in the period June 20 to August 24 were frozen until later autopsy. Feed samples from the farms were frozen until they could be analysed for mineral content.

The results showed that addition of fish silage through out the nursing period can reduce urinary pH in mink kits. Further it seems that the variations in feed composition between Danish feed kitchens do not influence urine pH in mink kits in June and July to the same extent as other factors. Mink type and genetic seems to be much more important for the incidence of diseases in the bladder. In this investigation there seems to be no obvious importance of management. The results from the mineral analyses will be reported later.

*Annual Report 2004, 215-222. Danish Fur Breeders Research Center, Holstebro, Denmark.*

### **Occurrence of coccidian in Danish farm mink submitted for diagnostic investigations**

*A.S. Hammer, T.H. Andersen, H.-H. Dietz*

A number of mink and faecal samples (n=307) submitted for diagnostic investigations January-October 2004, were examined for presence of endoparasites regardless of disease history and diagnosis. Furthermore, faecal samples were collected from mink (n=28) on three control farms without any current disease problems at the time of sampling (June). *Coccidia* oocysts were detected in faecal samples from 20.9 % of the mink included in this study (n=335). From two of the three control farms without current disease problem at sampling in June 2004 *coccidia* infections could be detected in 35-50% of the samples. The most frequent finding were *Isospora* spp. found in samples from 42 mink (12.5%). *Eimeria* spp. were found in samples from 25 mink (7.5%) and *Cryptosporidium* spp. were found in samples from four mink (1.2 %). The results confirm a high prevalence of *coccidia* infections among Danish farm mink submitted for diagnostic investigations. Though *coccidia* infections were found throughout the study period January-October (except for the month of April), the prevalence showed significant seasonal variation. The peak percentage of animals positive for *coccidia* was found in July, where 65 % of the examined samples were positive. When related to disease history and final diagnosis, the results did not imply that *coccidian* infections were a significant, primary cause of diarrhoea or other clinical disease in mink submitted for diagnostic investigations, but *coccidia*

could have some influence as a complicating factor to other diseases.

*Annual Report 2004, 223-228. Danish Fur Breeders' Research Center, Holstebro, Denmark.*

## **Reports on: Management**

### **Individual feed registration in the breeding period as a means of separating barren females (*Mustela vison*) – preliminary investigation**

*M. Sønderup, T.N. Clausen*

Using the program “individual feeding with Farmpilot” from Tved Maskinbyg, makes it possible to register feed ration on each cage at a mink farm. Daily feed ration per cage on a farm with 2500 breeding females in the period March 25 to April 8 2004 was registered. The purpose was to see if we could separate pregnant females from barren females by the amount of feed they consumed in the period from mating to mid April.

In the period from April 4 to April 8 barren females ate significant less feed than pregnant females. However a very big individual difference in feed consumption between females makes it impossible to isolate barren females at that time.

*Annual Report 2004, 229-233. Danish Fur Breeders' Research Center, Holstebro, Denmark.*

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