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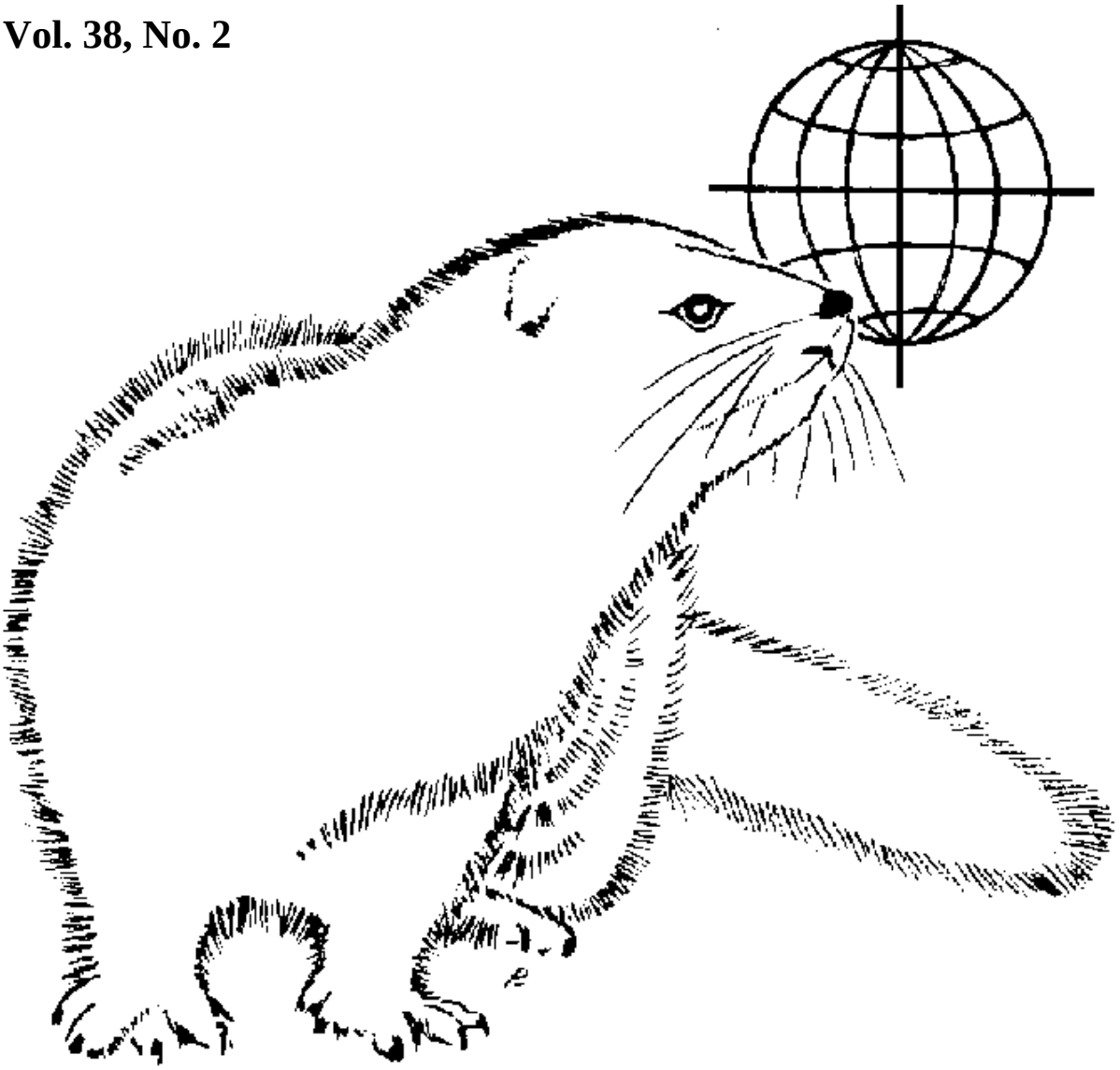

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Notes from the Editor

Improved resource efficiency is not only an issue for the production economy but also a condition for a future sustainable livestock production. Improved resource efficiency includes minimisation of the need for resources such as energy, N, P, and water in the production and prevention of avoidable wastes and emissions. This issue of Scientifur includes abstracts from the Annual Report 2013 from Kopenhagen Research at Kopenhagen Fur. Abstracts in the Annual Report reflect the large focus which is given to improved resource efficiency in the Danish mink production. Research is performed which examines the effect of reducing the protein content in the feed in the mating period, during pregnancy and lactation, and in the growing furring period. Results are also given from research examining the possibilities of reducing emission from the mink production.

A reviewed original paper dealing with resource efficiency is also published in Scientifur 38 2. In this paper, research is presented where the possibility of improving feed efficiency and nutrient digestibility by optimising dietary copper supplementation is studied.

A summary of a PhD thesis defended at Aarhus University, Denmark is given. In the study, the genetics of important production traits such as fur quality and litter size has been studied.

Attention should be given to:

- The 65th Annual Meeting of the European Association of Animal Production (EAAP), Copenhagen, Denmark 25-29 August 2014: http://www.eaap2014.org

- The Nordic NJF meeting, Grenå, Denmark 30 September to 3 October 2014. Further information can be obtained: tml@kopenhagenfur.com.

- The Abildgaard Seminar "Mink Health and Welfare", Copenhagen, Denmark 6-7 November 2014. Further information can be obtained: (hammer@sund.ku.dk).

Vivi Hunnicke Nielsen
Editor Scientifur

Effects of dietary copper supplementation on nutrient digestibility and growth performance in male mink

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Keywords: mink; copper; growth performance; nutrient digestibility

Abstract
The objective of this study was to study the effect of different levels of dietary copper on nutrient digestibility and growth performance of minks in the growing-furring periods. One hundred and five standard dark male minks were randomly assigned to seven groups with the following dietary treatments: basal diet with no supplemental Cu (Control); basal diet supplemented with either 6, 12, 24, 48, 96, and 192 mg/kg Cu from copper sulphate, respectively. Final body weight and average daily gain linearly (P=0.0026, P=0.0006) responded to increasing levels of Cu; maximal growth was seen in the Cu24 group. Feed efficiency tended to improve with the increase of dietary copper level (Linear P=0.0010, Quad,P=0.0011). Fat digestibility increased (linear and quadratic, $P < 0.05$) as Cu increased in the diet. Our results indicate that growing-furring mink can efficiently utilize added dietary fat and that Cu plays an important role in the digestion of dietary fat in growing-furring mink, and supplemental dietary Cu in growing-furring mink promotes fat digestion and improve growth performance.

Introduction

Copper, as an essential trace element, is required for plant, animal, and human health as well as for fur production of mink. Copper is a constituent of several metalloenzymes such as cytochrome oxidase, which functions in cellular respiration, and lysyl oxidase, important in connective tissue formation. Copper is also a constituent of tyrosinase, an enzyme involved in melanin (the black pigment of skin, hair, and fur). The recommended level for copper in the mink diet is 4.5-6.0 mg/kg (NRC, 1982). Danish mink feed normally contains 25-35 mg/kg copper (Leoschke, 2011). Studies by Mejborn (1989) with a control diet containing 5.1 mg/kg copper (wet weight basis) and experimental diets containing 39 and 116 mg/kg copper indicated no effect of copper supplementation on growth or fur production. However, earlier studies by Aulerich and Ringer (1976) indicated superior weight gains in male kits but not in female kits fed supplemental copper at the 50 mg/kg level. Studies at Cornell University with mink on purified diets suggested that 20 mg Cu/kg DM (dry matter) was adequate for growth and fur development (McCarthy et al., 1966).

Although copper has been found to play an important role in the nutrition and fur development of mink, the effects of copper supplementation on nutrient digestibility and fur quality of mink has never been reported. Furthermore, the optimal dietary level of Cu in minks has not been established. Therefore, the objective of the present study was to investigate the effect of dietary copper level on growth performance and digestibility of nutrients of mink in the growing-furring period. The optimum dietary copper requirement for mink was also estimated.

Materials and Methods

The animal protocol for this experiment was approved by the Animal Care Committee of the Institute of Special Economic Animal and Plant

Science of the Chinese Academy of Agricultural Sciences (44.02° N, 126.15° E). Animals were maintained and processed in accordance with the CAAS Guide for the Care and Use of Laboratory Animals.

One hundred and five standard dark male minks were randomly assigned to seven groups with the following dietary treatments: basal diet with no supplemental Cu (Control); basal diet supplemented with either 6(Cu6), 12(Cu12), 24(Cu24), 48(Cu48), 96(Cu96), and 192(Cu192) mg/kg Cu from copper sulphate, respectively. The experiment period lasted for 98 days. The composition and chemical analysis of the basal diet are shown in Table 1. The minks were weighted in the morning every two weeks before feeding from day 0 to the end of the trial. The left-over food was weighed and recorded daily. The ADG (average daily gain) and feed: gain was calculated for each animal individually.

On day 30 of the study, eight animals from each treatment group were selected randomly and housed individually in metabolic crates that allowed separation of urine and feces to determine nutrient digestibility and N balance, based on the method described by Jørgensen and Glem-Hansen (1973). The digestibility experiment lasted for 3 days and total feces and urine were collected and recorded. Feed were sampled for subsequent analysis. According the volume of urine, 10 ml 10% H_2SO_4 per 100 ml urine was used to combine the nitrogen and five drops of methylbenzene was used to prevent ammonia volatilization. The urine samples were collected using plastic bottles and stored at 20^0 C until they were analyzed. Fecal and feed samples were dried in a forced-air drying oven at 65^0 C, and then ground to pass the 40 mesh sieve. The apparent digestibility (AD) of nutrients and energy was calculated as follows:

$$AD = ((A - B)/A))x100\%.$$

A is the intake of nutrient from the diet and B is the nutrient in the feces. Data were analyzed using the General Linear Models (GLM) Procedure of SAS (2002). The following model was used:

$$Y_{ij} = \mu + d_i + \varepsilon_{ij},$$

Y_{ij} is the observation; μ is the general mean; d_i is the effect of Cu level (i = 1,…,7); ε_{ij} is the random error.

Tukey tests were used to detect statistical significance between treatment groups. Linear and quadratic effects due to copper level were determined. Significant differences were accepted if $P < 0.05$.

Results

Growth performance

No minks died during the experimental period. Effect of dietary copper levels on growth performance of minks is shown in Table 2. The initial body weight of the minks was similar among the treatments, however, final BW and ADG responded in a linear ($P < 0.05$) and quadratic ($P < 0.05$) fashion with increasing level of Cu; maximal growth was seen in the Cu24 group. The effect of Cu on ADFI (Average Daily Feed Intake) was linear ($P < 0.05$) and quadratic ($P < 0.05$); the highest ADFI was seen in the Cu192 group. Feed efficiency tended to improve with the increase of dietary copper level (Linear P=0.0010, Quad,P=0.0011)

Digestibility

Effect of dietary copper levels on apparent digestibility of nutrients is shown in table 3. There were no differences in apparent digestibility of DM, CP (crude protein) and Ash among the seven treatments ($P>0.05$). Fat digestibility increased (linear and quadratic, $P < 0.05$) as Cu increased in the diet.

Nitrogen balance

Effects of different dietary copper levels on nitrogen balance of mink are shown in Table 4. Daily nitrogen intake, fecal nitrogen, urinary nitrogen and nitrogen retention in animals were not significantly different among the treatment groups ($P > 0.05$).

Table 1 Composition and chemical analysis of the basal diet

Ingredient	**Contents [g/kg diet]**
Extruded corn	312
Soybean meal	60
Corn gluten meal	80
Fish meal	180
Meat and bone meal	180
Cheese meal	30
Soybean oil	120
Feather meal	10
Blood meal	10
Premix[a]	10
Lysine	3
Methionine	3
NaCl	2
Total	1000
Chemical composition (g/kg)	
Dry matter [g/kg]	976.0
Crude protein [g/kg DM]	330.5
Crude fat [g/kg DM]	167.2
Crude carbohydrate [g/kg DM]	424.1
Ash [g/kg DM]	60.2
Contents of mineral elements[a]	
Calcium [g/kg]	32.2
Phosphorus [g/kg]	22.0
Copper [mg/kg]	7.6
Zinc [mg/kg]	43.6
Iron [mg/kg]	203.4
Contents of amino acid[a] [mg/kg]	
Lysine	16916.4
Methionine	9260.3
Cysteine	3551.6
Metabolizable energy[b]	
MJ/kg DM	20.3
% from protein	30.6
% from fat	32.7
% from carbohydrates	36.7

[a]Contained the following per kg of premix：vitamin A, 1 000 000 IU; vitamin D3, 200 000 IU; vitamin E, 6 000 IU; vitamin B1,600 mg;vitamin B2, 800 mg; vitamin B6, 300 mg;vitamin B12 10 mg; vitamin K3,100 mg; vitamin C, 40 000 mg;niacin acid, 4 000 mg;pantothenic acid, 1 200 mg;biotin, 20 mg;folic acid, 80 mg;choline, 30 000 mg; Fe, 8 200 mg; Mn, 1 200 mg; Zn, 5 200 mg; I, 50 mg; Se, 20 mg; Co, 50 mg.
[b] Metabolizable energy was calculated according to Hansen et al. (1991)

Table 2 Effect of copper supplementation on growth performance of minks*

Item		Initial BW, g	Final BW, g	ADG, g	ADFI, g	Feed: Gain
Control		1393	1960^{bc}	6.30^{c}	113.12^{c}	19.66^{ab}
Cu6		1397	2133^{ab}	8.18^{ab}	112.90^{c}	16.52^{bc}
Cu12		1399	2042^{bc}	7.14^{bc}	114.06^{bc}	16.50^{abc}
Cu24		1397.74	2238^{a}	9.34^{a}	116.45^{abc}	12.90^{c}
Cu48		1401	2138^{ab}	8.19^{ab}	121.09^{a}	15.33^{bc}
Cu96		1394	2013^{bc}	6.88^{bc}	119.89^{abc}	18.54^{ab}
Cu192		1392	1931^{c}	5.99^{c}	120.80^{ab}	21.66^{a}
S.E.M †		9.08	18.69	0.17	0.69	0.53
p value	Linear	0.8682	0.0026	0.0006	0.0001	0.0010
	Quad	0.8754	0.0058	0.0013	0.0001	0.0011

*Values are means; Values not sharing the same superscript letters mean significant difference (P < 0.05), n = 15.
† SEM, standard error of the mean.

Table 3 Effect of copper supplementation on apparent digestibility of nutrient of minks*

Digestibility %		DM	CP	Fat	Ash
Control		66	72	85^{b}	33
Cu6		65	72	84^{b}	29
Cu12		67	72	87^{ab}	27
Cu24		66	73	88^{ab}	30
Cu48		68	72	87^{ab}	32
Cu96		65	71	88^{ab}	31
Cu192		8	72	90^{a}	28
S.E.M †		0.44	0.40	0.46	0.93
p value	Linear	0.2494	0.5223	0.0002	0.7840
	Quad	0.2425	0.5509	0001	0.6531

*Values are means; Values not sharing the same superscript letters mean significant difference (P < 0.05), n = 8.
† SEM, standard error of the mean.

Table 4 Effects of dietary copper levels on nitrogen metabolism in mink*

Item		Nitrogen intake (g/d)	Fecal nitrogen (g/d)	Urinary nitrogen (g/ d)	Nitrogen retention (g/d)
Control		5.40	1.69	3.52	0.20
Cu6		5.26	1.62	3.43	0.22
Cu12		5.09	1.60	3.30	0.19
Cu24		5.39	1.62	3.57	0.20
Cu48		5.38	1.65	3.51	0.22
Cu96		5.55	1.76	3.62	0.17
Cu192		5.46	1.71	3.55	0.21
S.E.M †		0.09	0.03	0.07	0.01
p value	Linear	0.2622	0.1939	0.3180	0.6213
	Quad	0.4148	0.3153	0.5008	0.8591

*Values are means; Values not sharing the same superscript letters mean significant difference (P < 0.05), n = 8.
† SEM, standard error of the mean.

Discussion

In our study, energy level in each group was designed to be kept consistent in order to eliminate the influence of energy. Hence, in this study, feed intake was mainly affected by the copper levels of diets. Feeding high levels of copper has been observed by a number of researchers to increase feed intake in other species (Edmonds et al., 1985; Burnell et al., 1988; Kornegay et al., 1989). However, to what extent Cu-stimulated feed intake contributing to growth stimulation is still an open question. Neuropeptide Y (NPY), an extremely potent stimulator of feeding behavior (King et al., 2000) may play an important role in regulating feed consumption. Intravenously injected copper has been shown to stimulate the secretion of neuropeptide Y (Zhou et al., 1994), which is a known feed intake stimulant for pigs. In our study, greater improvement in feed efficiency than in growth by copper supplementation has been observed. It is necessary to point out that an increase in feed intake in general will not only stimulate growth rate, but also improve feed efficiency, because the extra nutrients can be used almost exclusively for growth rather than for maintenance. Therefore, it is difficult to separate completely the contribution of increased feed consumption and feed efficiency to growth.

The apparent digestibility of crude protein and ether extract in this trial were slightly lower compared to values reported in other trials (Zhang et al., 2012; Ahlstrom and Skrede, 1998). This is most likely due to the composition of the diet, the fatty acids composition of the fat, or other dietary or environmental factors. Little is known about the effects of dietary copper on nutrient utilization in mink but information is available for pig. In the present study, fat digestibility was better in minks fed the high level copper diet compared with minks fed the other diet. Similar results have been reported in pigs (Dove, 1995; Luo and Dove, 1996). Luo and Dove (1996) reported that the addition of 250 mg/kg Cu improved digestibility and utilization of the fat in weanling pigs. The improved fat digestibility with copper supplementation may be due to copper enhanced the specific activity of the fatty acyl desaturase systems thus altering composition of depot fat. Moreover, the improvement in apparent fat digestibility due to copper addition observed in the present study and the previous study (Dove, 1995) may also be partially due to increased lipase and phospholipase A activities in the small intestine (Luo and Dove, 1996). Studies by Mejborn (1989) on copper sulfate supplementation of mink diets indicated that 75-90% of the copper intake was excreted in the feces and that urinary copper excretion was elevated with increased copper intake. This high excretion of copper can increase bile excretion (Harada et al., 1993; Gross et al., 1989). The primary function of bile is to emulsify fats in the small intestines and correspondingly to promote the fat digestibility. To our knowledge, this field of research is largely unexplored in this species of minks, and further investigations to better understand the relationships between fat digestibility and copper addition are warranted. Little is known about the effects of dietary copper on nitrogen balance in mink but information is available for pig. For example, Luo and Dove (1996) reported that copper supplementation significantly improved nitrogen retention in weanling pigs. However, our results showed that nitrogen retention was not affected by dietary Cu in mink.

Conclusions

The results of this feeding trial indicate that supplemental Cu plays an important role in the growth performance of mink. The data from this trial indicate that growing-furring mink can efficiently utilize added dietary fat and that Cu plays an important role in the digestion of dietary fat in growing-furring mink. Growing-furring mink seem to need dietary Cu in excess of levels normally found in mink diets for the optimum utilization of dietary fat.

Acknowledgments

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Faglig Årsberetning

2013

Kopenhagen Forskning

Annual Report

2013

Kopenhagen Research

Influence of timing of moving mink dams after mating on maternal stress, maternal care and early kit survival

J. Malmkvist

Mink dams build and maintain a nest at least 1 month prior to delivery, in case of access to abundant nest building material. The timing of moving mated dams from mating cages to delivery cages do not affects the visible size of the nest after the delivery or the growth in surviving kits. However after delivery, late moved dams had colder nests than nests of early moved dams. Late transfer tended to increase the mortality among litters affected by early mortality. Fewer kits from early transferred dams vocalise when away from the female, tested day 5 after birth. Dams moved around April 10th had around 50% higher concentration of the hormone cortisol during the period prior to delivery; this higher concentration was not due to a higher number of kits, and thus indicative of stress. In case females are to be moved, transfer just after mating is preferable, as negative effects were present following later times of movement, in particularly in case the move takes place around April 10th.

Annual Report 2013: 7-18. Kopenhagen Research, Denmark

Group selection against bite marks in group housed juvenile mink is efficient, but unknown environmental factors are also important

S.H. Møller, S.W. Hansen

A selection experiment have demonstrated a high heritability for bite marks in mink, if the indirect social effects between group housed mink is included in the selection. The number of bite marks was, however, not reduced in the group housed mink selected for few bite marks, while the number increased in the unselected control line. Thus, other factors are also important in practice. We tested the hypothesis that early separation of mink kits into group housing reduces the number of bite marks compared to late separation. The hypothesis was tested at the research farm in Foulum and at a private farm simultaneously. We reject the hypothesis as the date of separation had no clear effect on the number of bite marks in juvenile mink. The expected effect was seen in the females but not in the males at Foulum, while the opposite effect was seen in males on the private farm, where no effect was seen in females. Therefore the search for influential management and environmental factors that affect the number of bite marks in mink continues.

Annual report 2013: 19-24. Kopenhagen Research, Denmark

We can breed for more social mink!

P. Berg, S.W. Alemu, S.H. Møller, L. Janss, P. Beijma

Social interactions are common in animals and may affect many important production traits. As the number of group housed mink increases, social interactions such as aggression, has become increasingly important to investigate. Researchers from Aarhus and Wageningen University have tested the hypothesis that a larger part of the total variation in bite marks is genetic when social interactions are included as indirect genetic effects. The investigation shows that bite marks scored in the scraped skin at pelting is largely due to indirect genetic effects caused by social interactions between mink in the group. The sum of direct and indirect genetic effects explained a large part of the variation in bite marks. Therefore, mink that receive fewer bites and bite their cage mates less can be efficiently selected.

Annual report 2013: 25-28. Kopenhagen Research, Denmark

Reduced protein to mink kits in the growing furring period 2012

T.N. Clausen, P.F. Larsen

Continued studies on protein and amino acid requirement in the growing-furring period in brown and black mink. Five groups of black mink (150 males and females) and 7 groups of brown mink (175 males and females) were used for the study. The protein content varied between 32 and 24 MEp (metabolisable energy from protein) with or without addition of synthetic amino acids or glucose. The feed registration showed only small variations in the uptake of calories when the protein content was

reduced, but a marked reduction in protein uptake. Reducing protein from 32 MEp in the whole period to 30 MEp from August 10 and further to 24 MEp from mid-September, reduced protein uptake by 8 – 9 per cent, and had no negative effect on skin size and quality. A further reduction in protein to 26 MEp from August 10 and 24 MEp from mid-September, reduced protein consumption by 13 – 15 per cent, but also a tendency towards reduced skin quality. Fatty liver increased with decreasing protein content in the feed in the low protein groups.

Annual Report 2013: 29-40, Kopenhagen Research, Denmark.

Blue whiting – a new raw material for mink feed

M. Engbæk, L. Tinggaard, P.F. Larsen

The palatability and digestibility of blue whiting was determined by two trials. The palatability trail showed that the whole blue whiting can be used as raw material in mink feed, without affecting the taste and feed intake. Results from the digestibility trial showed good agreement between values found for the tested blue whiting and values listed in the table of feed ingredients for Industrial fish with less than 5% fat. The protein and fat digestibility was respectively 89% and 97% for blue whiting, and 88% and 96%, respectively, for industrial fish. It is recommended that Blue whiting is listed as an individual raw material in the table of feed ingredients.

Annual Report 2013: 41-46. Kopenhagen Research, Denmark.

Reduced protein to mink females in the mating period

T.N. Clausen, P.F. Larsen

To investigate female mink's requirement of protein in the mating period, we used 5 groups of 300 brown females and 3 groups of 167 black females. The females were fed the same feed except in the period from February 22 to April 6, were the protein content varied from 30 to 50 MEp in the different groups.
No significant negative effect of reducing feed protein content in the mating period to 30 MEp in brown females and to 35 Mep in black females was observed.

Annual Report 2013: 47-54, Kopenhagen Research, Denmark.

Selection of mink that manage well on a low protein content in the feed. Status for growing-furring period 2012 and breeding period 2013

T.N. Clausen, P.F. Larsen

It is very important that mink are healthy and perform well on the fed we produce, and it is necessary to select the animals that perform best under the given conditions. In July 2011 two selection groups that will be followed for several generations were started. One control group fed a protein level corresponding to the level at Danish feed kitchens in 2009 and one fed a 15% protein reduction compared to the 2009 level.
In the second growth period (2012) males in the low protein group had shorter skins and a lower skin quality compared to the control group. The females in the low protein group had a lower weight but a better skin quality. It is possible that problems in the previous nursing could have had an impact on the results.
In the second reproduction period (2013) litter size in the low protein group were higher than in the control group, probably not due to the feed composition but more likely due to differences in the body conditioning for the females in the two groups in the winter period.

Annual Report 2013: 55-66, Kopenhagen Research, Denmark

Effects of nutritional supplements on blood and liver parameters and health in growing-furring mink

B.M. Damgaard, P.F. Larsen, V.M. Thorup, T.N. Clausen

The purpose of the project was to investigate the possibility of preventing the negative effects of low protein diets on health by nutritional supplements to a low protein diets in growing-furring mink. The experiment included investigation of body weight, mortality rate and clinical-chemical parameters in

blood and liver. Three groups of each 175 male and female brown mink were included in the study. One control group was fed high dietary protein content during the growing-furring season (KON). For one group the dietary protein content was reduced in August and September to 24 % of metabolizable energy from protein (MEp) and the diet was supplemented with dextrose (GLU). One group received from mid-August low dietary protein content (20 MEp) (LP) and this group was at mid-September divided in 7 subgroups that received different dietary supplements. All LP-groups received from mid-October high dietary protein content. The frequency of dead animals with fatty liver was high at low dietary protein content in October. Nutritional supplements to a low protein diet could not prevent high mortality rate and fatty liver at low protein diets. The liver seemed to be able to regenerate after fat infiltration when fed high protein diet during one month. High dietary protein content seemed to have positive effects on performance and health. Nutritional supplements to a low protein diet could not prevent the negative effects of a low protein diet on growth and health.

Annual Report 2013: 67-76. Kopenhagen Research, Denmark

The effect of reduced protein content in the feed on the plasma metabolic profile in the growing-furring period

M.S. Hedemann, B.M. Damgaard, T.N. Clausen, P.F. Larsen

The purpose of the present investigation was to study the effect of two protein reduction strategies compared to a control protein level on the composition of metabolites in plasma in mink. In one strategy (Low protein 1) the protein level was reduced from 32 to 24 % of metabolizable energy (MEp) and in the other (Low protein 2) the protein level was reduced from 28 to 20 % MEp during the growing-furring period. Blood samples were collected during the experimental period (August-November). The metabolic profile differed between blood samples taken in August and November. Some of the metabolites causing this were tryptophan and phenylalanine that were observed in higher concentration in August compared to November and an unidentified metabolite observed in samples from November, but absent in August. When data from October was analyzed separately it was observed that mink fed 20 % MEp formed one group and mink fed 24 % and 28 % MEp formed another group. Some of the metabolites responsible for the separation were tryptophan, phospholipids (high in control and “Low protein 1”) and phenylalanine (high in “Low protein 2). The metabolic changes observed in the present study have also been found in humans with metabolic disorders like obesity, diabetes and metabolic syndrome.

Annual Report 2013: 77-84. Kopenhagen Research, Denmark

Recovery and stability of amino acids in mink feed stored at different temperatures

M. Engbæk, P.F. Larsen

Loss and degradation of added synthetic amino acids can potentially be a problem in mink feed. The stability of synthetic amino acids was investigated in mink feed when stored at 5°C, 20°C and 35°C during a period of three days.
Results showed a mean recovery of approx. 52% of synthetic amino acids just after incorporation, though with variation between the different amino acids. A high stability of added amino acids was observed over time when the feed was stored at 5°C, and a degradation of the amino acids over time when stored at respectively 20°C and 35°C.

Annual Report 2013: 85-90. Kopenhagen Research, Denmark

Does drinking water pH influence water intake in mink?

M. Engbæk, L. Tinggaard, P.F. Larsen

Mink’s preference over the water's acidity was tested at Kopenhagen Farm. 20 male mink were given the choice between water with pH value 7.6 and 6.3, respectively. The duration of the trial was four weeks. The trial showed no significant difference regarding water type or water intake. The acidity of the drinking water also did not influence the urine output or the pH-value of the urine. However there were indications that the mink had a preference towards the placement of the water bottle

and not so much the content in the pH-range investigated in this study. The difference in acidity may have been too small for the mink to notice, and thereby having no practical effect.

Annual Report 2013: 91-96. Kopenhagen Research, Denmark

Effect of additional water supply in late lactation

T.N. Clausen, P.F. Larsen

To optimise weaning and increase kit welfare the effect of additional water in the nest box from dag 28 to day 56 was investigated in a group of brown mink kits. The results showed that adding water increased body weight in male and female kits from day 28 to 42, and for females the effect lasted until day 56. No difference in number of mink kits with bites was observed among the two groups.

Annual Report 2013: 97-102. Kopenhagen Research, Denmark

Low protein provision in the gestation during several generations in mink

C.F. Matthiesen, A.-H. Tauson

An overall reduction in protein content in mink feed may affect the mink production in both a short- and long-term perspective. Our objectives were to investigate how low protein provision during late gestation affects the reproductive performance, kit survival and pre- and post-weaning growth performance of F_1, F_2 and F_3-generation offspring of protein restricted mothers raised in small (4-6 kits) or large (8-10 kits) litters until weaning. The reproductive performance was not significantly affected by the gestation diet, but the results indicate that dams exposed to a low protein provision during fetal life and fed a low protein diet during late gestation gave birth to larger litters and had fewer stillborn kits than adequately treated dams fed a low protein diet during late gestation. The kit birth weight of offspring born by mothers fed a low protein diet during late gestation was significantly ($P<0.05$) lower than controls which confirms previous results. The growth rate of the kits during lactation indicated that offspring exposed to low protein provision during fetal life performed better in large litters than adequately treated ones especially in the transition period from milk to solid feed and around weaning ($P<0.05$).

Annual Report 2013: 101-108. Kopenhagen Research, Denmark

The level of protein provision to mink dams before implantation affects fetal survival rate

C.F. Matthiesen, A.-H. Tauson

The protein and amino acid requirements are still not completely known in all parts of the mink production cycle. According to Danish legislation there is a demand to reduce the overall nitrogen emission and it is therefore important to investigate whether the levels of protein commonly used today can be reduced. Our objective was to investigate and determine the protein requirement before implantation needed to support a good reproduction performance. Six different levels of protein (20, 25, 30, 35, 40 and 45% of metabolisable energy - ME- from protein) were fed to 96 female mink from the 24th of February until the 10th of April. Three females from each treatment were euthanized the 16th of April to investigate the implantation rate, fetal survival, fetus length and weight. The remaining dams were used to measure the reproductive performance. The number of implanted fetuses was not affected by the protein provision whereas the survival rate of implanted fetuses was significantly ($P=0.02$) lower in the group fed the 20% of ME from protein compared to the other groups. The protein requirement before implantation, estimated by a broken line linear regression approach from the fetus survival rate, indicate that the requirement was 30.5% of ME from protein. The reproductive performance was not inferior in the group fed 25% compared to 30% and 35% of ME from protein. Inexplicably was however, there were a significantly higher number of barren dams and a tendency towards increased kit losses in the group fed 30% of ME from protein compared to other groups.

Annual Report 2013: 109-116, Kopenhagen Research, Denmark

Shaking mink syndrome

M. Chriel, M.S. Hansen, E. Holm, G. Larsen, C.K. Hjulsager, H.L. Enemark, T.K. Jensen

Shaking mink syndrome has previously been described in Denmark in a few farms. The syndrome is characterized by uncoordinated movements and the symptoms gradually worsen. Since the animals cannot coordinate eating behaviour they will die due to starvation if not killed. In the previous outbreaks the symptoms were only observed in July in only a few young mink kits. In 2013, a massive outbreak in one farm was noted. In May, approximately 13% of the litters in the farm had diarrhoea and were treated against preweaning diarrhoea. Shaking mink syndrome was recorded in more than 300 mink and in contrast to earlier cases the adult mink were also affected.

Annual Report 2013: 117-120. Kopenhagen Research, Danmark

The connection between outbreaks of canine distemper virus in Danish farmed mink and distemper in free-ranging terrestrial carnivores

L.G. Albrechtsen, L. Andresen, L., Nielsen, T. Struve, C.R. Olesen, S. Bilk, J.F. Agger, A.S. Hammer

Distemper in farmed mink is caused by canine distemper virus (CDV). In 2012 an unusually high number of distemper outbreaks occurred on Danish mink farms and a total of 64 mink farms were diagnosed with distemper. The aim of this project was to assess the prevalence of distemper in the Danish free-ranging terrestrial carnivores. A total of 266 animals were tested for distemper, including red fox, Eurasian badger, beech marten and free-ranging American mink. Seven foxes tested positive for distemper, primarily from the southern part of Jutland. Sequence analysis showed a high degree of similarity between the distemper positive samples and one distemper sample from a farmed mink from the outbreaks of 2012, which cannot exclude the possibility that the virus has spread from wild foxes to farmed mink and vice versa. Furthermore the field isolates were similar to German CDV strains. So typing results, timeline and geographical distribution of outbreaks indicates that that the virus has spread to Denmark from German wildlife.

In collaboration with Kopenhagen Fur a questionnaire-based survey of biosecurity measures was completed. The survey included 51 distemper-free farms and data from 51 distemper-positive farms involved in the distemper outbreaks of 2012 and early 2013. The results indicate that both farms accessed by free-ranging foxes and farms with unvaccinated animals were predisposed for distemper outbreaks. Moreover it was clear that biosecurity measures varied within both groups (cases and controls).

Annual Report 2013: 121-128, Kopenhagen Research, Denmark

Investigation of parvo-, corona-, and astrovirus as causative agents in outbreaks of diarrhea in Danish mink kits in the nursing and growth period 2013 - Preliminary results

S. Hansen, L.I. Krarup, J.F. Agger, K. Ullman, K.O. Hedlund, J. Klingström, L. Andresen, A.S. Hammer

Mink diarrhea can be linked to economic loss within the mink fur production and it is one of the most important reasons for antibiotic treatment of mink. In the attempt to make the diagnostic options more easily available and affordable this study aims at developing multiplex PCR assays, which may be applied in both routine diagnostic work and research of virus related diarrhea in mink. During a period from May to November 2013 a total of 216 mink from farms with outbreaks of diarrhea have undergone necropsy. Fecal and tissue samples have been collected for further diagnostics by histopathology, bacteriology and electron microscopy. Fecal samples have been tested by qPCR for detection of mink parvo virus, corona virus and astro virus. A high number of astrovirus positive farms were seen in May and June, in particular on farms with lesions characteristic of “greasy kits”. No cases of parvovirus have been detected in the submitted animals. Samples from May, June and July were corona virus negative, the rest of the samples remains to be tested.

Annual Report 2013: 131-138. Kopenhagen Research, Denmark

The effect of provision of water in the nest box on the occurrence of cutaneous wounds and mortality of farmed mink kits (*Neovison vison*)

A. Jespersen, A.S. Hammer, J.F. Agger, H.E. Jensen

Studies of methods for protection against cutaneous wounds in mink are important due to animal welfare and economy in mink production. Here we present results from a study of the effect of providing water bottles in the nest box on wound occurrence and mortality in mink kits during the lactation period.
This study was performed on a commercial mink farm, on which 5850 mink kits from 886 litters of colour types Brown/Glow and Pearl were followed daily from 10th of June until weaning. Every other litter received drinking water in a water bottle directly in the nest box.
Providing water by bottle in the nest box reduced the occurrence of wounds and mortality among the mink kits in the late lactation period. There were found more dead kits from litters without water bottles than with water bottles. The most frequent causes of death were diarrhea (sticky kits syndrome), dehydration and/or emaciation and wounds.

Annual Report 2013: 139-146. Kopenhagen, Denmark

Healing of wounds in farm mink (*Neovison vison*)

D. Mogensen, T.N. Clausen, A. Jespersen, J.F. Agger, A.S. Hammer

In 2013 the healing of 55 wounds was examined in order to assess the healing process by various treatments. Today there is very little available data on wound healing in mink. The primary objective of this pilot study was to collect data on wound healing in mink, which can form the basis for the planning of more extensive field studies and experimental studies of wound healing and wound treatment in mink. In addition, the aim was to evaluate different types of treatment. In June mink were treated with wound sprays with or without antibiotics. In October-November wounds in one group was treated with wound spray containing antibiotics and wounds in another group was treated with bath in 1.5 % saline solution followed by wound spray without antibiotics.
Most of the wounds healed quickly and without complications and within a three weeks period. In June wounds on the ear and wounds on the side of the body healed more slowly than wounds on the shoulder and neck. Wounds in October-November were located at the base of the tail and the wounds healed fast and uncomplicated during the study period.

Annual Report 2013: 147-154. Kopenhagen Research, Denmark

Pathological investigations of the reproduction organs in male mink (*Neovison vison*) and associations with litter size – preliminary results

A.S. Hammer, B. Christensen, J.F. Agger, H. Elvang-Jensen, T.N. Clausen

The purpose of this investigation was to evaluate the occurrence of gross lesions in the reproductive organs in the male mink and to evaluate associations with reproduction results. Testes and baculum (and penis) from a total of 121 male mink pelted on Kopenhagen Farm during the month of March 2013 were collected and examined. Weight and measures of testes and baculum and body weight of the animals were recorded and compared with the willingness to mate and the litter size. In this investigation we found a positive correlation between size of testes and litter size and a positive correlation between testes size and willingness to mate. In this study, 1.7% of the animals were diagnosed with chryptorchidism (undecended testes), and atrophy of the epididymis was not diagnosed in any of the mink. It was not possible to show an association between size of baculum and size of testes, body weight or colourtype, but the results indicate an association between size of baculum and reproduction results. Causal factors affecting the development of the baculum in male farm mink are currently unknown. The results of this study indicate that there is need for further investigation of causal factors of poor reproduction results in male mink.

Annual Report 2013: 155-160. Kopenhagen Research, Denmark

Examination of histological lesions in an experimental mink astrovirus immunization study

M.S. Hansen, B. Claudia, U. Karin, T.H. Jensen, G. Larsen, M. Chriél

Astrovirus infection in mink kits is associated with the syndrome "greasy kits" that causes diarrhea and often results in major losses in the mink production. Therefore, there is great interest in developing vaccines that can reduce the disease burden and the associated economic losses. In the described experiment two astrovirus vaccines, based on the C1 and C4 capsid proteins, were tested. Fifteen pregnant mink dams were immunized with one of the vaccines and their puppies were subsequently inoculated with astrovirus to evaluate the induction of immune responses, and the development of symptoms and tissue lesions. The immunizations with particular the C1 vaccine reduced the duration and severity of clinical symptoms, histological lesions and viral shedding.

Annual Report 2013: 163-170. Kopenhagen Research, Denmark

Extensive outbreak of canine distemper in Danish farmed mink (*Neovison vison*) and wild carnivores

R. Trebbien, M. Chriel, T. Struve, C.H. Hjulsager, G. Larsen, L.E. Larsen

A major outbreak of canine distemper virus (CDV) in Danish farmed mink (*Neovison vison*) started in the late summer period of 2012. At the same time, a high number of diseased and dead wildlife species such as foxes, raccoon dogs, and ferrets were observed. To track the origin of the outbreak virus full-length sequencing of the receptor binding surface protein hemagglutinin (H) was performed on 26 viruses collected from mink and 10 viruses collected from wildlife species. Subsequent phylogenetic analyses showed that the virus circulating in the mink farms and wildlife were highly identical with an identity at the nucleotide level from 99.45 % to 100 %. The sequences could be grouped by single nucleotide polymorphisms according to geographical distribution of mink farms and wildlife. The outbreak viruses clustered phylogenetically in the European lineage and were highly identical to wildlife viruses from Germany and Hungary (respectively 99.29% and 99.62%). The study documented for the first time that fleas (*Ceratophyllus sciurorum*) contained CDV and that vertical transmission of CDV occurred in a wild ferret. The study provides evidence that wildlife species, including foxes, play an important role in the transmission of CDV to farmed mink and that the virus may be maintained in the wild animal reservoir between outbreaks.

Annual Report 2013: 171-178. Kopenhagen Research, Denmark

Technical effect of acidification on mink slurry

M. Engbæk, M.N. Hansen, S.G. Rasmussen, K.H. Meldgaard, H., Bækgaard, P.F.Larsen

Kopenhagen Fur has, in collaboration with Hyldgaard Staldservice and AgroTech, conducted a study to investigate the technological effect on acidification of mink slurry on ammonia emission. The study compared the ammonia emission from 72 mink, housed in a climate-controlled research stable, equipped with a prototype of an acidified chamber system, and traditional slurry channels (reference system).

Air flow and ammonia emissions were continually determined in the research stable. Results showed that the ammonia emissions per mink in the acidification period were reduced by 85% compared to the average ammonia emission found during the reference period. The experiment with the acidified slurry also showed a consistency between the ammonia emission and the pH-value of the slurry. The higher pH, the higher evaporation. In the period after the slurry was acidified, there was a gradual increase in the slurry pH. In order to maintain a low pH-level in the slurry, the slurry should periodically be re-acidified.

Annual Report 2013: 179-184. Kopenhagen Research, Denmark

Source separation of mink manure – a technology to reduce ammonia emission

K. Meldgaard, M.N. Hansen, M. Engbæk, H. Bækgaard, P.F. Larsen

The effect of source separation of mink manure was tested at Kopenhagen Farm, to study its potential to

reduce ammonia emission. For source separation, the urine fraction was drained to a closed container, and thereby separated from the solid fraction of the manure. The result from the source separation method was compared to a reference method, where both the manure and urine fraction was collected in slurry channels. The study showed that the source separation method could reduce ammonia emissions between 85% to 95% compared to the reference method, when the temperature ranged between 15 - 20 degrees Celsius and using weekly cleaning of the slurry channels. In addition, no increase of ammonia was found over time when using source separation compared to the reference method. During the reference periods an increase in the ammonia concentration occurred around day three. The study also showed that the ammonia emission was affected by temperature.

The study indicates that there is a potential in the development of new environmental technologies to separate the mink manure, and thereby reducing the ammonia emissions from the slurry channels in the mink production.

Annual Report 2013: 185-192. Kopenhagen Research, Denmark

Bite marks on the leather side of mink pelts can be added by mechanical pressure

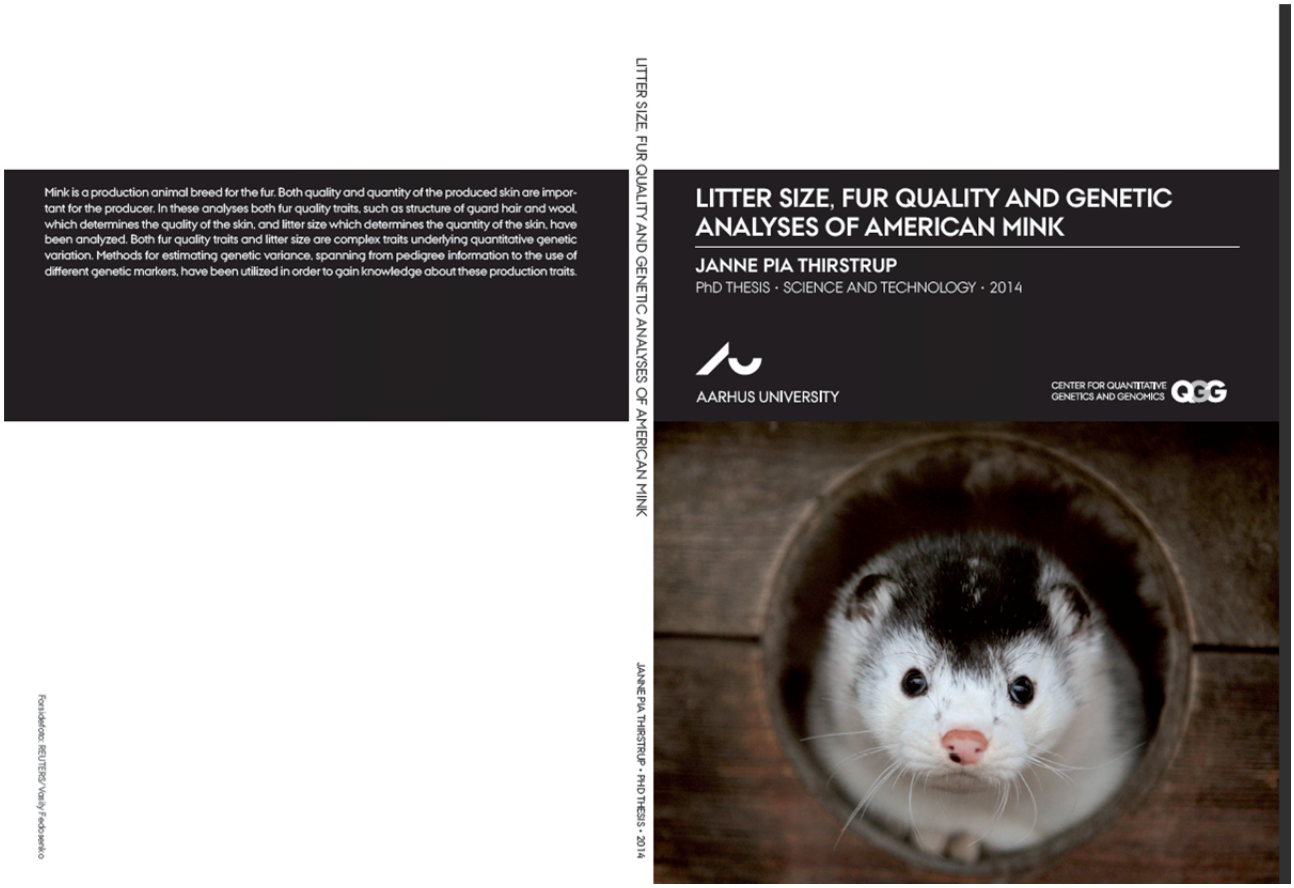
S.W. Hansen, S.H. Møller, B.M. Damgaard

It has been questioned whether bite marks on the leather side of the skin are caused by bites. Alternatively, bite marks have been interpreted as a spot wise delayed maturation of the winter coat unrelated to the level of aggression. Therefore, we tested the hypothesis that experimentally applied pressures/bites to the mink skin, during the growth phase of the winter coat, will produce bite marks that can be recognized as such at pelting. Furthermore, we also tested that the longer time mink are kept in groups, the more bite marks can be observed on the skin. The results from the study show that mechanical pressure on the skin can be recognized as bite marks in dark coloured mink at pelting. Furthermore, were we able to demonstrate that the number of bite marks increases with the time mink is kept in groups. The results indicate that aggression or other social contact is the cause of bite marks.

Annual report 2013: 193-198. Kopenhagen Research, Denmark

Litter size, fur quality and genetic analyses of American mink

PhD thesis by Janne Pia Thirstrup



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2014

The genetics behind traits important for fur production has been explored during a three-year Ph.D. project. The traits in question were litter size and fur quality traits. In recent years, discovery of genetic markers in general has developed very fast. New types of genetic markers and new methods for analyzing DNA are constantly evolving. During the Ph.D. project it has been possible to utilize different types of genetic markers to explore production traits in mink.

In the first part, heterosis and genetic variance components for litter size were analyzed. Pedigree and litter size data from three mink farms using crossbreeding strategies were available. Because of their different crossbreeding strategies it was possible to analyze a broad spectrum of crossing combinations. Evidence for maternal effect was found, meaning that it is more important that breeding females themselves are products from crossbreeding compared to the importance of their offspring being products from crossbreeding. It was also found that selection can be effective for improving litter size in mink.

In the second part, quantitative trait loci (QTL) important for fur quality traits were identified. For the analyses, a population of three generations produced from crossing Nordic Brown and American Black short nap was used. More than 100 microsatellite DNA markers and a genetic linkage map, developed for the purpose, were used. At least two different chromosome areas for each trait were identified. Some of the identified areas for single traits coincided and it was suggested that guard hair length and guard hair thickness are affected by the same genes or by genes in close proximity.

In the third part, it was investigated if DNA from mink could be amplified using a predesigned single nucleotide polymorphic chip (SNP-chip) created from the dog genome. This is a novel technique that has previously been used for several species. One hundred and eleven SNPs from the dog SNP-chip amplified successfully using mink DNA, and it was confirmed that this method can be used as a cost effective and fast method for generating genetic resources for mink.

In the final part, Next-Generation Sequencing (NGS) was used for the purpose of generating a panel of SNPs that can be used for population genetic analyses in American mink. Farm mink were RAD-sequenced and from these sequences almost 225,000 polymorphic loci was identified in these mink. After strict filtering processes primers were designed for 380 SNPs. The panel was tested on a large sample of both farm and feral mink from Denmark and Poland. The panel proved to have high resolution for inferring population structure and population assignment.

The Ph.D. project has provided insights in traits important for mink production and the results can be used by mink breeders and scientists working in mink genetics. Genomic resources for mink have been developed during this project and the work provides a stepping stone for further development of genomic resources for mink.

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