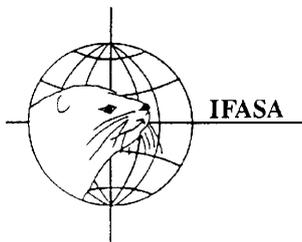
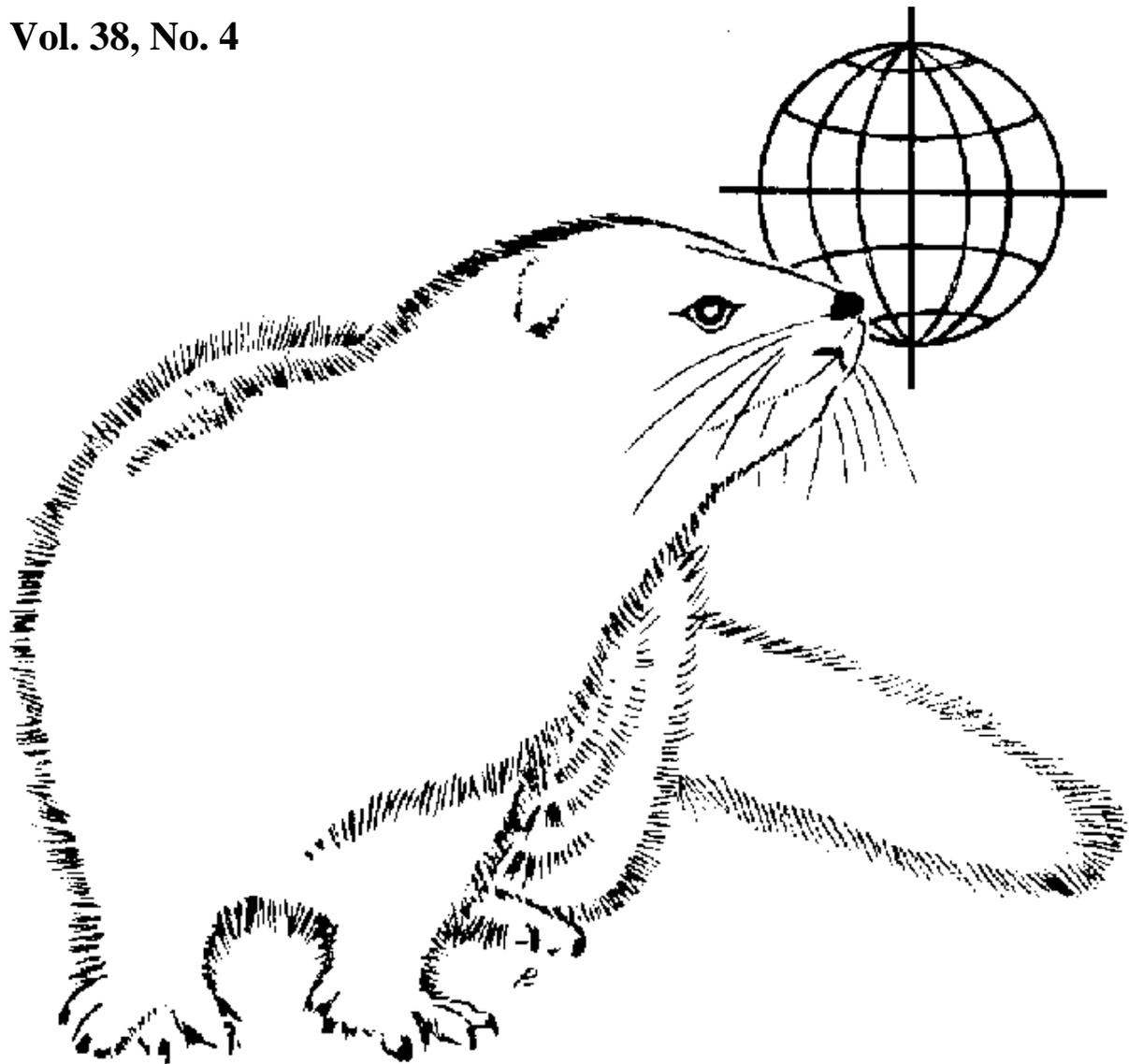


SCIENTIFUR

SCIENTIFIC INFORMATION IN FUR ANIMAL PRODUCTION

Vol. 38, No. 4



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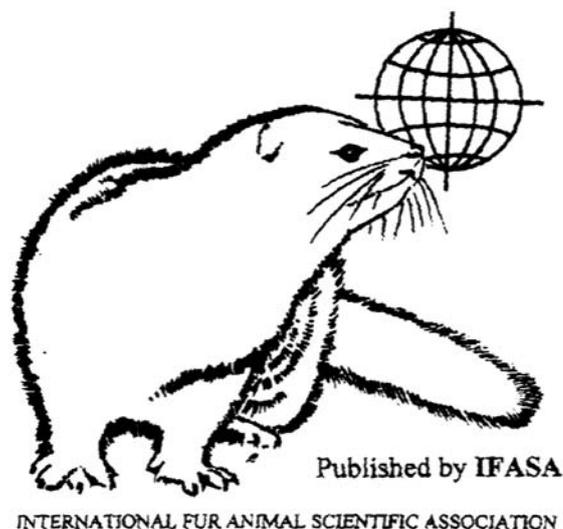
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Notes from the Editor

In 2004, the European Commission initiated the “Welfare Quality” project focusing on the three main livestock species: cattle (beef and dairy), pigs, and poultry (broiler chickens and laying hens). The research project was designed to develop European standards for on-farm welfare assessment and product information systems as well as practical strategies for improving animal welfare.

In 2009, the European Fur Breeders Association (EFBA) launched the welfare program “WelFur” inspired by the European “Welfare Quality” project. The objective of the “WelFur” program is to develop a general certification protocol at European farm level, which will guarantee a high level of animal welfare on fur farms. Protocols for both mink and foxes have now been developed in a scientific programme consisting of a consortium of 7 European universities and research institutes. The assessment protocols are based on both scientific and commercial farm tests.

The implementation of “WelFur” consists of 4 pillars:

1. The publishing of the “WelFur” assessment protocols
2. The creation of a software tool to calculate the scores and store the data.
3. The development of training protocols and materials for the assessors
4. National implementation action plans

Results from the “Welfare” welfare assessments were presented at the Nordic NJF meeting in Denmark in the autumn 2014. Abstracts from the meeting are given in this volume of *Scientifur*.

Litter size is a complex character determined by many different traits both in the female and male parent and in the foetuses. Maintaining or improving litter size in fur animal production requires focus on these different traits. Abstracts presented in this volume of *Scientifur* deal with improvement of litter size. Although the heritability for litter size in mink is low as also observed in other species it is recommended to improve litter size by selection. The same study shows also that crossing of lines within the same colour type is a mean to improve litter size. In silver foxes, flow cytometry is shown to be a useful tool to evaluate fresh semen ejaculates for artificial insemination. More basic studies of semen characteristics in blue fox are also presented.

Attention should be given to the yearly Nordic NJF meeting which will be held in Åbo in Finland from 29 September to 1 October 2015. Further information can be obtained at tml@kopenhagenfur.com.

Vivi Hunnicke Nielsen
Editor *Scientifur*

BREEDING, GENETICS AND REPRODUCTION

Heterosis and genetic variation in the litter size of purebred and crossbred mink

J.P. Thirstrup, P.F. Larsen, C. Pertoldi, J. Jensen

Crosses between different mink lines from 3 Danish mink farms that use different breeding strategies were studied to estimate heterosis and variance components for litter size. The study was designed to analyze crosses between lines of the same color type, between different color types, and between animals originating from different farms. Effect of heterosis, color type, and variance components were estimated using Average information REML (AI-REML) algorithm implemented in the DMU package for analyzing multivariate mixed models. Females from 7 generations that gave birth to at least 1 offspring were analyzed and the effects of parity and production year were included in the analyses. Genetic trend and the proportions of the total variance explained by the effects of additive genetics (h^2), common environment (due to repeated litters from the same female; c^2), and dam of the female (granddame of the born litter; d^2) were estimated. The results showed that mink of the Black color type potentially produced smaller litters compared to mink of the other studied color types. We found significant general maternal effect of heterosis for litter size. Analyses of specific heterosis showed a significant positive effect of crossing between lines of the same color type. Estimates of variance components revealed h^2 levels for farm A, B, and C of 0.15, 0.06, and 0.09, respectively; thus litter size could be selected for in the future. The effect of common environment on litter size was also considerable, with c^2 values of 0.005, 0.11, and 0.15 at farms A, B, and C, respectively. In conclusion, we recommend genetic selection as a means of increasing litter size in farmed mink.

J. Anim. Sci. 2014: 92(12): 5406-5416
doi: 10.2527/jas.2014-7781 [Epub 2014 Nov 17]

<http://www.ncbi.nlm.nih.gov/pubmed/25403189>

A base substitution in the donor site of intron 12 of KIT gene is responsible for the dominant white coat colour of blue fox (*Alopex lagopus*)

S.Q. Yan, J.N. Hou, C.Y. Bai, Y. Jiang, X.J. Zhang, H.L. Ren, B.X. Sun, Z.H. Zhao, J.H. Sun

The dominant white coat colour of farmed blue fox is inherited as a monogenic autosomal dominant trait and is suggested to be embryonic lethal in the homozygous state. In this study, the transcripts of KIT were identified by RT-PCR for a dominant white fox and a normal blue fox. Sequence analysis showed that the KIT transcript in normal blue fox contained the full-length coding sequence of 2919 bp (GenBank Acc. No KF530833), but in the dominant white individual, a truncated isoform lacking the entire exon 12 specifically co-expressed with the normal transcript. Genomic DNA sequencing revealed that a single nucleotide polymorphism (c.1867+1G>T) in intron 12 appeared only in the dominant white individuals and a 1-bp ins/del polymorphism in the same intron showed in individuals representing two different coat colours. Genotyping results of the SNP with PCR-RFLP in 185 individuals showed all 90 normal blue foxes were homozygous for the G allele, and all dominant white individuals were heterozygous. Due to the truncated protein with a deletion of 35 amino acids and an amino acid replacement (p.Pro623Ala) located in the conserved ATP binding domain, we propose that the mutant receptor had absent tyrosine kinase activity. These findings reveal that the base substitution at the first nucleotide of intron 12 of KIT gene, resulting in skipping of exon 12, is a causative mutation responsible for the dominant white phenotype of blue fox.

Anim. Genet. 2014: 45(2): 293-296
doi: 10.1111/age.12105. [Epub 2013 Dec 6]

<http://www.ncbi.nlm.nih.gov/pubmed/24308634>

Setting Priorities for Existing Conservation Needs of Crayfish and Mink

M. Díez-León, R. Miranda, A.H. Ariño, D. Galicia

Conserv. Biol. 2014
doi: 10.1111/cobi.12406 [Epub ahead of print]
<http://www.ncbi.nlm.nih.gov/pubmed/25308514>

The complete mitochondrial genome sequence of Neovison vison (Carnivora: Mustelidae)

W.L. Sun, S.J. Wang, Z. Wang, H.L. Liu, W. Zhong, Y.H. Yang, G.Y. Li

The phylogenetic and taxonomic position of the American mink *Neovison vison* have long been unclear. In this paper, the complete mitogenome of *N. vison* was sequenced and characterized. The total length was 16,594 bp and typically consists of 37 genes, including 13 protein-coding genes, 2 rRNAs, 22 tRNA, a large control region (CR) and a light-strand replication origin (O_L). Gene contents, locations, and arrangements were identical to those of typical vertebrate. The overall base composition is 33.6%, 25.4%, 27.8% and 13.3% for A, C, T and G, respectively, with a moderate bias on AT content (61.4%). This result is expected to provide useful molecular data and contribute to further taxonomic and phylogenetic studies of Mustelidae and Carnivora.

Mitochondrial DNA 2014: 1-2. [Epub ahead of print]

<http://www.ncbi.nlm.nih.gov/pubmed/25319300>

Flow cytometric evaluation of sperm apoptosis in semen of silver foxes in the breeding period

K. Kostro, L. Krakowski, U. Lisiecka, A. Jakubczak, A. Zmuda, P. Wojtaszczyk, A. Waschocka

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doi: 10.1016/j.anireprosci.2013.10.006. [Epub 2013 Oct 26]*

<http://www.ncbi.nlm.nih.gov/pubmed/24332010>

Kinematics and subpopulations' structure definition of blue fox (*Alopex lagopus*) sperm motility using the ISAS® V1 CASA system

C. Soler, A. García, J. Contell, J. Segervall, M. Sancho

Over recent years, technological advances have brought innovation in assisted reproduction to the agriculture. Fox species are of great economical interest in some countries, but their semen characteristics have not been studied enough. To

advance the knowledge of function of fox spermatozoa, five samples were obtained by masturbation, in the breeding season. Kinetic analysis was performed using ISAS® v1 system. Usual kinematic parameters (VCL, VSL, VAP, LIN, STR, WOB, ALH and BCF) were considered. To establish the standardization for the analysis of samples, the minimum number of cells to analyse and the minimum number of fields to capture were defined. In the second step, the presence of subpopulations in blue fox semen was analysed. The minimum number of cells to test was 30, because kinematic parameters remained constant along the groups of analysis. Also, the effectiveness of ISAS® D4C20 counting chamber was studied, showing that the first five squares presented equivalent results, while in the squares six and seven, the kinematic parameters showed a reduction in all of them, but not in the concentration or motility percentage. Kinematic variables were grouped into two principal components (PC). A linear movement characterized PC1, while PC2 showed an oscillatory movement. Three subpopulations were found, varying in structure among different animals.

*Reprod. Domest. Anim. 2014: 49(4): 560-567
doi: 10.1111/rda.12310. [Epub 2014 Jun 2]*

<http://www.ncbi.nlm.nih.gov/pubmed/24890953>

Effect of age and breeding season on sperm acrosin activity in the arctic fox (*Alopex lagopus* L.)

K. Stasiak, B. Janicki

The objective of this study was to determine the effect of age and reproductive season on selected properties of semen from the arctic fox, *Alopex lagopus* L. The experiment used 40 ejaculates collected manually from 6 animals (3 foxes aged one year and 3 foxes older than three years). Statistically less semen (0.39 cm³) was collected from the young compared to the older animals, and the ejaculates obtained were characterized by higher concentration of spermatozoa (195.04 x 10⁶/cm³). In turn, sperm acrosomal extracts from the older animals contained statistically more acrosin (6,4 mU/10⁶ spermatozoa). In the sperm acrosomal extracts prepared during the first semen sampling, the mean acrosin activity did not exceed 2.3 mU/million spermatozoa. At subsequent semen

sampling dates, the activity of the analysed enzyme increased to reach 7.72mU/million spermatozoa. In the extracts obtained from the semen collected at the end of the breeding season of arctic foxes, the acrosin activity again reached a value obtained at the beginning of the season.

Polish Journal of Veterinary Sciences 2014: 17(1) 177–179

BEHAVUOUR AND WELFARE

Benefits of a Ball and Chain: Simple Environmental Enrichments Improve Welfare and Reproductive Success in Farmed American Mink (*Neovison vison*)

R.K. Meagher, J.A. Dallaire, D.L. Campbell, M. Ross, S.H. Møller, S.W. Hansen, M. Diez-León, R. Palme, G.J. Mason

Can simple enrichments enhance caged mink welfare? Pilot data from 756 sub-adults spanning three colour-types (strains) identified potentially practical enrichments, and suggested beneficial effects on temperament and fur-chewing. Our main experiment started with 2032 Black mink on three farms: from each of 508 families, one juvenile male-female pair was enriched (E) with two balls and a hanging plastic chain or length of hose, while a second pair was left as a non-enriched (NE) control. At 8 months, more than half the subjects were killed for pelts, and 302 new females were recruited (half enriched: 'late E'). Several signs of improved welfare or productivity emerged. Access to enrichment increased play in juveniles. E mink were calmer (less aggressive in temperament tests; quieter when handled; less fearful, if male), and less likely to fur-chew, although other stereotypic behaviours were not reduced. On one farm, E females had lower cortisol (inferred from faecal metabolites). E males tended to copulate for longer. E females also weaned more offspring: about 10% more juveniles per E female, primarily caused by reduced rates of barrenness ('late E' females also giving birth to bigger litters on one farm), effects that our data cautiously suggest were partly mediated by reduced inactivity and changes in temperament. Pelt quality

seemed unaffected, but E animals had cleaner cages. In a subsidiary side-study using 368 mink of a second colour-type ('Demis'), similar temperament effects emerged, and while E did not reduce fur-chewing or improve reproductive success in this colour-type, E animals were judged to have better pelts. Overall, simple enrichments were thus beneficial. These findings should encourage welfare improvements on fur farms (which house 60-70 million mink p.a.) and in breeding centres where endangered mustelids (e.g. black-footed ferrets) often reproduce poorly. They should also stimulate future research into more effective practical enrichments.

PLoS One 2014: 9(11): e110589
doi: 10.1371/journal.pone.0110589

<http://www.ncbi.nlm.nih.gov/pubmed/25386726>

HEALTH AND DISEASE

MicroRNA miR-320a and miR-140 inhibit mink enteritis virus infection by repression of its receptor, feline transferrin receptor

J.Z. Sun, J. Wang, S. Wang, D. Yuan, Z. Li, B. Yi, Q. Hou, Y. Mao, W. Liu

Mink enteritis virus (MEV) is one of the most important pathogens in the mink industry. Recent studies have shed light into the role of microRNAs (miRNAs), small noncoding RNAs of length ranging from 18–23 nucleotides (nt), as critical modulators in the host-pathogen interaction networks. We previously showed that miRNA miR-181b can inhibit MEV replication by repression of viral non-structural protein 1 expression. Here, we report that two other miRNAs (miR-320a and miR-140) inhibit MEV entry into feline kidney (F81) cells by downregulating its receptor, transferrin receptor (TfR), by targeting the 3' untranslated region (UTR) of TfR mRNA, while being themselves upregulated.

Virol J. 2014: 11(1) 210 [Epub ahead of print]

<http://www.ncbi.nlm.nih.gov/pubmed/25465595>

Eenie, Meenie, Miney, Moe, who is responsible for the antibody-dependent enhancement of Aleutian mink disease parvovirus infection?

H.W. Zhu, X.M. Xing, Y.J. Wen

Aleutian mink disease parvovirus (AMDV) causes a persistent infection associated with immune complex disease, hypergammaglobulinemia, and high levels of antiviral antibodies. Despite the presence of an antibody, the virus is not cleared *in vivo*. Pre-existing antibodies may enhance viral infections, by Fc-receptor-mediated antibody-dependent enhancement (ADE), but the mechanism that underlies ADE has not been fully defined. Three models have been proposed, including: (1) interactions between antibody and FcR, complement C3 fragment and CR, or between C1q and C1qR, which promotes viral attachment to cells; (2) suppression of IFN-gamma-mediated host-cell antiviral gene expression by the upregulation of negative regulators of pathogen pattern recognition; and (3) the promotion of early IL-10 secretion. In addition, the role of cytokine IL-6 in ADE mediated disease development is discussed, to facilitate a better understanding of the pathogenesis of AMDV infection, as well as give insights into rational vaccine design approaches.

Bing Du Xue Bao 2014: 30(4): 450-455

<http://www.ncbi.nlm.nih.gov/pubmed/25272602>

Asthma among mink workers

B. Grøntved, O. Carstensen, R. Petersen, D. Sherson

We report two cases of asthma among mink workers. The first case is about a mink farmer who had asthma that was difficult to treat. In the medical history there was no clear relation to work, and no conclusive work relation with peak flow monitoring. He had a positive histamine release test to mink urine. The second case is about a mink farm worker, who had an asthma attack when handling mink furs. Peak flow monitoring showed a clear relation to this work, but there were no signs of allergy. We conclude that these two cases suggest an increased risk of asthma among mink workers.

*Ugeskr. Laeger 2014: 176(40)
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Haemorrhagic enterotoxemia by *Clostridium perfringens* type C and type A in silver foxes

Ł.S. Jarosz, Z. Grądzki, A. Śmiech, M. Kalinowski

Type C and type A of *C. perfringens* were detected in the seat of natural infections in silver foxes characterized by symptoms of haemorrhagic enterotoxemia. In all of the dead foxes characteristic changes were noted in the small intestine and parenchymatous organs. The production of *alpha* and *beta* toxins by isolated bacteria was confirmed by the bioassay using white mice and by PCR. The results of the drug sensitivity testing showed that isolated strains were highly susceptible to amoxicillin with clavulanic acid, metronidazole, doxycycline and penicillin with streptomycin.

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Characterization of a new epidemic necrotic pyoderma in fur animals and its association with *Arcanobacterium phocae* infection

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A new type of pyoderma was detected in Finnish fur animals in 2007. The disease continues to spread within and between farms, with severe and potentially fatal symptoms. It compromises animal welfare and causes considerable economic losses to farmers. A case-control study was performed in 2010-2011 to describe the entity and to identify the causative agent. Altogether 99 fur animals were necropsied followed by pathological and microbiological examination. The data indicated that the disease clinically manifests in mink (*Neovison vison*) by necrotic dermatitis of the feet and facial skin. In finnraccoons (*Nyctereutes procyonoides*), it causes painful abscesses in the paws. Foxes (*Vulpes lagopus*) are affected by severe

conjunctivitis and the infection rapidly spreads to the eyelids and facial skin. A common finding at necropsy was necrotic pyoderma. Microbiological analysis revealed the presence of a number of potential causative agents, including a novel *Streptococcus* sp. The common finding from all diseased animals of all species was *Arcanobacterium phocae*. This bacterium has previously been isolated from marine mammals with skin lesions but this is the first report of *A. phocae* isolated in fur animals with pyoderma. The results obtained from this study implicate *A. phocae* as a potential causative pathogen of fur animal epidemic necrotic pyoderma (FENP) and support observations

that the epidemic may have originated in a species-shift of the causative agent from marine mammals. The variable disease pattern and the presence of other infectious agents (in particular the novel *Streptococcus* sp.) suggest a multifactorial etiology for FENP, and further studies are needed to determine the environmental, immunological and infectious factors contributing to the disease.

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NJF SEMINAR 482

**AUTUMN MEETING
IN FUR ANIMAL RESEARCH 2014**

30 SEPTEMBER - 3 OCTOBER 2014

KYSTVEJENS HOTEL AND CONFERENCE CENTER

GRENAA - DENMARK

WELFUR SESSION

A study on foot welfare in foxes

H.T. Korhonen, P.Eskeli, T. Lappi, J. Sepponen

The aim was to clarify effects of ad libitum and restricted feeding with three Ca:P ratios on foot welfare in blue foxes. Experiment was started at weaning on August 6th (wk 32) and finished at pelting on November 26th (wk 47). Treatment groups were: (1) restricted feeding (R 1), Ca:P ratio 1.5:1; (2) restricted feeding (R 2), Ca:P ratio 2.9:1; (3) restricted feeding Ca:P-ratio control level (R 3); (4) ad libitum feeding (AL 1), Ca:P ratio 1.5:1; (5) ad libitum feeding (AL 2), Ca:P-ratio 2.9:1; (6) ad libitum feeding (AL 3) Ca:P ratio control level. Body growth variables, feed consumption and several welfare variables were measured. The behaviour of animals was video recorded. Foreleg carpal joint angle as an indicator of leg weakness and the fox's ability to move about in the cage were evaluated. During weeks 32-35 and 37-47 animals from restricted groups ate 60- 65% and 67-68% of the given feed, respectively. Body weight gain fed ad libitum foxes was faster. In Oct 3, ad libitum groups significantly differed from restricted feeding groups ($P<0.001$). At pelting, waistline and neck circumference was significantly ($P<0.001$) larger in ad libitum groups compared to restricted ones. Also body condition score (BSC) was significantly ($P<0.001$) larger for ad libitum animals. Body length was greater for ad libitum than for restricted groups ($P<0.05$). Significant differences were not found in body size variables between Ca:P- ratios. During the course of experiment bending of feet increased in each group. This increase between initial and final scoring was significant ($P<0.001$). Most bended feed was found at last scoring on Nov 18. Significant differences were found in bending between ad libitum and restricted groups ($P<0.05$). Pronounced difficulties in locomotor activity were not found. Differences between the groups were slight in stick, floorball test and capture test. No withdrawal or pain reaction was found at sensitivity test. Foot welfare problems seem to be a multi-factorial problem that requires further research.

Implementation of the WelFur welfare assessment: results from Finnish fox farms

L. Ahola, T. Koistinen, J. Mononen, H. Huuki

In this presentation, we show the WelFur assessment results from Finnish fox farms assessed in the national Fur Farm 2020 project. Altogether 14 persons were trained for the WelFur fox farm assessments. Seven out of these made more than two farm assessments. In addition to this, four researchers from the WelFur project and/or the Fur Farm 2020 project took part in the assessments, assessing approximately 25% of all the assessments. Altogether 248 farm visits on 88 separate fox farms (corresponding approx. 10% of the Finnish fox farms) were carried out during autumn 2012 - winter 2014. Most of the farms were assessed three times, according to the WelFur fox protocol. However, ten out of the farms were assessed only one or two times, mainly due to some farmers' opt-outs from the project. The assessment results show that, in general, the incidence of undesirable findings was relative low on most of the farms, i.e. the farm level median of observed undesirable findings - like diseases, abnormal behaviour and deficiencies in the resources given for foxes - was most often zero. The results show that most commonly observed problems on Finnish fox farms were bent feet, diarrhoea and fatness. All these problems were observed especially in blue foxes. Furthermore, our experiences from practising the implementation of WelFur suggest that more emphasis should perhaps be put on the training of the assessors, and on the post-training support for assessors due to possible observer drift.

Social interactions in pair and group housed mink

L. Olofsson, S.W. Hansen, L. Lidfors

The study was conducted on a private farm from September-November in brown mink kept in pairs (one male, one female, $n=12$ cages) or in groups (one male, two females, $n=12$ cages). Cages consisted of a lower (0.281 m^2), and upper floor (0.142 m^2), a nest box. Direct observations of the frequency of biting behaviour, wrestling and chasing another mink were done during one day for 5 weeks in two periods 1.5 h around sunrise and

before lunch. Observation from the video were done one hour before sunrise every fourth week for three observations. Data for direct observations was analysed per animal with Generalised Linear Model and for video with Proc Glimmix mean \pm SE are shown. There were no differences between treatments in biting behaviour (11.11 ± 1.29 , $p=0.7813$), wrestling (7.56 ± 1.08 , $p=0.1500$) and chasing (0.85 ± 0.20 , $p=0.7658$). Concerning the presence of social interactions from video, being in the nest or out in the cage, there were no significant differences between the two treatments, pairs, social (175 ± 44), out in cage (1541 ± 128), in nest (1778 ± 180) or groups, social (187 ± 42), out in cage (1214 ± 108), in nest (1999 ± 153). Quick bite and mating was performed very few times that it was not possible to test this statistically. In conclusion there was no difference in the occurrence of social interactions between pair and group housing found in this study.

The WelFur-mink classification differs between the three annual production periods

B.I.F. Henriksen, S. H. Møller, J.T. Sørensen

According to the WelFur-assessment protocol for mink we need to assess welfare at three one day visits, one in each of three main annual production periods. If one of these three assessments could provide a sufficient representative description of the welfare on a mink farm throughout the year we will save time and money and provide the farmer with an immediate result. As the manager is usually the same in all three periods and as most welfare problems in mink production are related to management we hypothesize that the level of welfare will be highly related between the three periods assessed. Based on the WelFur-assessment protocol for mink, data from 19 farms was collected in each of the three production periods: Breeders during winter; Females and kits in lactation during summer; Juveniles during late growth in autumn. The data was recorded by two external assessors per farm, on 9 farms in 2011 and on 10 other farms in 2013, and an aggregated welfare assessment at farm level was calculated for each of the three visits. Data from the three assessments per farm was compared at principle level of welfare. The results of the study indicate that the WelFur principle of “Good feeding” can be predicted from the seasonal score

value from the summer period and the principle of “Good housing” can be predicted from both the seasonal score value from the winter and summer period. The full annual principle of “Good health” and “Appropriate behaviour” could not be predicted from a single period. Based on the present results we cannot accept the general hypothesis that one of the seasonal scores of the principle of welfare of a farm in the welfare assessment system WelFur can represent the full annual assessment across all three periods. This shows that the different welfare challenges in the three periods have great influence on the welfare assessed by WelFur despite the constant factor of farm management. Further analysis is needed to evaluate the possibility of using seasonal score-values to predict the full annual principle of “Good feeding” and “Good housing”.

First step to implement WelFur registrations on Danish mink farms

B.K. Hansen, M. Blæsbjerg-Obitsø, A.F. Marsbøll, E. Mortensen, A.M. Grauman

Welfare assessments will be conducted on 15 Danish mink farms in the period 2014-15. The aim of the project is to prepare implementing of the WelFur assessment protocol in a large scale on commercial farms and to answer some further questions: Is the variation among commercial farms in Denmark visible in the registration methods? Is it necessary to take the variation within the observation periods (January-February) and (October-November) into account in the final welfare score? Another challenge is the feasibility of the registrations as there are only three observation periods a year of each 6-8 weeks. How do we make the registrations as efficient as possible without compromising with validity and reliability of the observations? Experiences and results from observation period one will be presented.

Effects of different nesting materials to mated mink

J. Malmkvist

The early kit survival day 0-3 after delivery is one of the critical points, determining the potential of increasing the number of kits produced per

delivering female in the mink production. Previous studies have documented that birth problems (influenced by e.g. female body condition and nest building materials) increase the risk of early kit mortality, reduced maternal care and kit vitality. The mated females are motivated to build a nest prior to delivery. Nesting materials of an insufficient nature (e.g. wood shavings only) increase the stress hormone concentration in the dams and reduce kit survival. In contrast, several other types of nesting materials, given in the pre-delivery period, reduce stress and improve kit survival. In a new study we compare six different combinations of nesting material to mated yearlings, either as simultaneously Free Choice (FC) or as a prepared Mixture (M): 1: FC of straw, wood wool, and rabbit wool (N=60), 2: FC of straw, wood wool, and lamb's wool (N=60), 3: FC of straw, lamb's wool (N=60), 4: M, straw with 2.5 % lamb's wool (N=60), 5: M, straw with 10 % lamb's wool (N=60), and 6: straw (N=60), all groups with *ad libitum* access out in the cage from the time of transfer of the dam to the maternity (after mating March 25) until 7 days after delivery. The data analysis is on-going, and selected preliminary results on nest building (scored weekly), kit survival and growth (day 1-7) and dam stress hormone concentration measured non-invasively (faeces cortisol metabolites before and after delivery) will be presented and discussed.

The area of the nest box affects group housed mink's use of the nest box during daytime

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

There is strong evidence for mink having a behavioral need for access to a nest box (Hansen, 1988; Hansen et al., 1994, 1998; Møller 1990). Lack of access to nest box increases the incidence of abnormal behavior, increases the physiological stress levels, reduces growth and fur quality and increases kit loss during lactation. However, there is no clear evidence of which size of nest box that best meets needs of the mink in different situations. Studies have shown that mink in groups choose to "huddle" together in a single nest box and do not use other available nest boxes for resting (Hagn, 2009). This indicates that mink seek social contact and tolerate limited space in the nest box. Haagensen & Jeppesen (2007) examined three different sizes of nest boxes and found that 2-3 mink often lay together in a standard nest box, but divided into two

nest boxes if housed four or more together. Nest boxes of 80% of standard size were used the least and nest boxes of 120% of the standard size were used the most. The result indicates that the space in the nest box can limit the mink's use of the nest box. The aim of this experiment was to test the hypothesis that mink will spend more time in nest boxes with a large area (L:36 x W:29 x H:13,5) than in nest boxes with a small area (L:26 x W: 29 x H: 18,5), both having the same volume (approx. 15500 cm³). Furthermore, we tested whether the number of bite marks was affected by the area of the nest box.

NUTRITION SESSION

Water for mink in late lactation

T.N. Clausen and P.F. Larsen

Animal total water consumption is dependent on ambient temperature, feed composition, sodium chloride content in the feed, digestibility, dry matter and feed energy concentration. Lactating females are especially loaded in the fourth week of lactation where the milk production is up to 200 ml per day, and for mink kits the drinking water system when they start to drink is very important. Drip water system or rabbit water bowls in late lactation can be of benefit for the kits, their body weight increase is improved and one study has demonstrated less bite among mink kits. We have focused on adding fibers or Feed binders to increase the water content in the feed and make it easier for the kits to fulfill their need of water. Results show that the choice of fiber is important for improving the water balance for the mink kits and so far the perfect fiber has not been found.

Breath test measurement of the utilisation of ¹³C-labelled L-leucine and L-methionine in male mink (*Neovison vison*) during intensive fur growth - Effect of prenatal and post-weaning protein provision

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

The utilization of the ¹³C-labelled crystalline amino acids L-leucine and L-methionine in male mink was evaluated by means of breath test measurements.

Twelve 25 weeks old male mink born by dams that had been given either an adequate (FA; n=6) or a low (FL; n=6) protein supply in late gestation were used. The animals were given either an adequate (AP) or low (LP) protein provision after weaning, thus with three animals on each of the four treatment combinations (FAAP, FALP, FLAP, FLLP). The recovery of ^{13}C after an intraperitoneal injection of ^{13}C -labelled amino acid was measured in two periods by means of breath test measurements. Each period lasted six days during which daily quantitative collection of faeces and urine was performed. Each period included a 22-h respiration experiment (indirect calorimetry) and breath test measurements of ^{13}C recovery of L-leucine (period 1) or L-methionine (period 2). The ratio between $^{13}\text{C}/^{12}\text{C}$ in samples of air drawn from the respiration chambers every third minute was analysed by Isotope Ratio Infrared Spectroscopy (IRIS) and rates of instantaneous and cumulative recovery were calculated and expressed as percentage of the ^{13}C -dose. The rate of recovery of labelled CO_2 in expired air as a percentage of injected dose increased steeply shortly after injection and was apparent already after 3 minutes for both leucine and methionine. Measurements showed that methionine reached peak values and declined towards baseline values more rapidly than leucine. Cumulative recovery was significantly lower for methionine ($6.7 \pm 1.8\%$) than for leucine ($21.9 \pm 1.8\%$) of the injected dose. It was concluded that male mink utilized the crystalline forms of the two amino acids L-leucine and L-methionine in the metabolism. Methionine recovery was lower, suggesting that crystalline methionine was used in protein metabolism to a higher extent than crystalline leucine during the furring period.

Metabolism of natural and synthetic vitamin E in mink kits and their interaction with vitamin A and D

S.K. Jensen, T.N. Clausen, P.F. Larsen

A well balanced vitamin supplementation is a prerequisite for a good growth and wellbeing of mink. Fat soluble vitamins are most sensitive towards over or under supply, especially because they interact with each other with respect to dose and chemical form. The present paper comprises two experiments. The purpose of the first experiment was to investigate the effect of

increasing amount of vitamin A (0-6000 IU/kg feed) and either synthetic vitamin E or natural vitamin E (100 mg/kg feed) to growing mink on their vitamin A, D and E status. The feed was supplemented with 700 IU vitamin D_3 /kg feed. Two forms of vitamin E was used (synthetic *all-rac- α -tocopheryl acetate* and natural RRR- α -tocopherol (Immun E[®] Natur)). After a vitamin A + E depletion period from mid July to mid August this experiment was performed from mid August to mid September. The experiment showed that mink already in the unsupplemented feed met their vitamin A requirement. Further the experiment showed that mink did not face a negative effect of large amounts of vitamin A on vitamin E status as pigs and calves. However, the experiment showed that vitamin D status in plasma decreased with increasing vitamin A in the feed. Also natural vitamin E in the feed decreased plasma vitamin D status compared to synthetic vitamin E. However feeding natural vitamin E had a positive influence on vitamin A status in the kidneys. Generally female had higher vitamin status than the males.

The purpose of the second experiment was to elucidate the difference in bioefficiency in weaned mink kits of the three commercial available vitamin E sources. This study was undertaken due to the importance of this vitamin and its high cost in mink feed. Thus, it is relevant to elucidate the relative bioefficiency of the expensive natural vitamin E on alcohol form (RRR- α -tocopherol) and the cheaper natural acetate form (RRR- α -tocopheryl acetate) against the cheapest form, synthetic vitamin E (*all-rac- α -tocopheryl acetate*). Twelve groups of 12 mink, 4 week of age were allocated to 4 levels of vitamin E (50, 75, 100 or 150 mg/kg feed) of one of the 3 types of vitamin E, in addition a vitamin E free group served as control. After 3 weeks on the experimental diets, six mink were sacrificed and plasma, liver, heart, lung and brain was removed, weighed and subsequent analysed for vitamin E content and stereoisomer composition. This procedure was repeated with 6 new mink again after 6 weeks. After 3 and 6 weeks on the experimental diets, α -tocopherol concentration in plasma and tissue was significant higher in mink fed natural vitamin E compared to synthetic vitamin E and dose response curves for the 3 types of vitamin E showed significant differences in non-linear regression curves for plasma and tissues. However, until 75 mg/kg α -tocopherol the two natural sources gave same response, at higher concentrations in the feed the alcohol form was superior to the acetate form.

Likewise the biodiscrimination of the different stereoisomers varied between the different tissues, especially the liver retains the majority of the synthetic stereoisomers since they are not active transported into circulation in the blood and further out into the other tissues. RRR- α -tocopherol showed the highest bioefficiency and synthetic all-rac- α -tocopherylacetat the lowest bioefficiency.

Dietary supplements to a low protein diet may affect the incidence of hepatic lipidosis in mink

C.F. Matthiesen, A-H. Tauson

Hepatic lipidosis, i.e. hepatic lipid infiltration, is multifactorial and may be caused by a number of factors such as low protein provision, feed deprivation, rapid accretion or mobilisation of body fat, all resulting in metabolic and nutritional imbalances. Our objectives were to investigate if supplementation of a low protein diet with nutrients acting as methyl donors, antioxidants or having insulinogenic properties could lower the incidence of hepatic lipidosis in growing mink from August to November when mortality, caused by hepatic lipid infiltration, often is high.

Seventy-two young mink, 36 females and 36 males, were used in the study. The animals were allocated to six treatment groups each consisting of 6 males and 6 females. The control group was fed a conventional farm feed (29.6:54.2:16.2 % of metabolisable energy (ME) from protein: fat: carbohydrate) whereas the 5 experimental groups were fed a low protein diet (20% of ME from protein, prepared by adding 5.5% corn starch, 3.5 corn oil, 6% potato mash powder, 1% sugar beet pulp and 8% water to the control diet) and supplemented with the crystalline amino acids alanine (0.8% - negative control - group 1), taurine (0.5% - group 2), arginine (0.5% - group 3), and methionine (0.5% - group 4) or the carbohydrate dextrose (2.5% of the corn starch was replaced with dextrose - group 5). Feed intake was recorded as an average per group per day. The animals were weighed and blood sampled once every third week. The liver was removed and the liver and body weights were recorded of all animals that died during the experiment. All remaining animals were euthanized at the end of the experiment when liver, kidney and body weights were recorded. All livers were frozen for later analysis. Liver weight was significantly affected ($P < 0.001$) by dietary

treatment, status (dead or alive) ($P < 0.001$) and interactions between dietary treatment and status ($P < 0.001$). This resulted in significantly heavier livers among animals diagnosed with hepatic lipidosis compared to livers from euthanized animals without hepatic lipidosis. The survival rate was numerically higher for control (males 100%, females 100%) and group 4 (methionine – males 100%, females 100%) animals compared to group 1 (alanine – males 84%, females 100%), group 2 (taurine – males 84%, females 100%), group 3 (arginine – males 84%, females 84%) and group 5 (dextrose – males 67%, females 84%) animals. It can be concluded that our preliminary results indicate that the methionine level in a low protein diet plays an important role for the incidence of hepatic lipidosis.

Status on protein optimization in Danish mink production 2014

P.F. Larsen, T. Clausen

During the last years the protein content in Danish mink feed has been lowered in a cost-effective/economic and environmental perspective in order to optimize the protein use during the mink production year. Many years of intense research in both universities and Kopenhagen Furs research farm, combined with practical experience and use from feed kitchens, have resulted in general recommendations to the Danish feed kitchens on the minks need for protein and other nutrients throughout the production year. This presentation will provide an update on the status on protein optimization in the Danish mink production and the challenges based on research activities from Kopenhagen Farm. Finally it will also present and discuss future strategies for protein optimization in Denmark.

Low protein provision during the first year of life, but not during foetal life, affects metabolic traits, organ mass development and growth in male mink (*Neovison vison*)

A-H. Tauson

Low protein provision *in utero* and *post-partum* may induce metabolic disorders in adulthood. Studies in mink have mainly focused on

short-term consequences of low protein provision *in utero* whereas the long-term responses to low protein (LP) provision in metabolically programmed mink are unknown. We investigated whether low protein provision *in utero* affects the long-term response to adequate (AP) or LP provision after weaning in male mink. Eighty-six male mink were exposed to low (19% of ME from CP; crude protein) or adequate (31% of ME from CP) protein provision *in utero*, and to LP (~20% of ME from CP) or AP (30–42% of ME from CP) provision post-weaning. Being metabolically programmed by low protein provision *in utero* did not affect the response to post-weaning diets. Dietary protein content in the LP feed after weaning was below requirements; evidenced by lower nitrogen retention ($p < 0.001$) preventing LP mink from attaining their growth potential ($p < 0.02$). LP mink had a lower liver, pancreas and kidney weight ($p < 0.05$) as well as lower plasma IGF-1 concentrations at 8 and 25 ($p < 0.05$) weeks, and a higher incidence of hepatic lipidosis at 25 weeks ($p < 0.05$). Furthermore, LP mink had a higher body fat ($p < 0.05$) and lower body CP content ($p < 0.05$) at 50 weeks of age. It is concluded that some effects of low protein provision *in utero* can be alleviated by an adequate nutrient supply *post-partum*. However, long-term exposure to low protein provision in mink reduces their growth potential and induces transient hepatic lipidosis and modified body composition.

Metabolic programming by means of low protein provision *in utero* modifies the metabolic response to diet in mink (*Neovison vison*): A proteomic approach

L. Anjos, A.C. Guerreiro, K. Vesterdorf, C.F. Matthiesen, D.M. Power, A.P. Harrison, A-H. Tauson

Metabolic programming *in utero* has long-lasting consequences for human health, however, the consequences for mink kits and their ability to adjust post-natally remains unknown. This study has addressed the issue of fetal life protein restriction on the liver proteome of mink kits at 20, 25 and 50 weeks of age compared to kits provided an adequate diet during. Furthermore, the potential restorative effects of an adequate diet from weaning to 50 weeks of age were investigated. Four dietary treatment groups were established; fetal low (FL) or fetal adequate protein (FA) provision, followed

post-weaning by either low (LP) or adequate protein (AP) provision. Protein restriction during fetal did not affect liver- or body composition, however, the liver proteome was affected. Significant ($P < 0.05$) changes in the abundance of proteins involved in glycolysis (eg: TPI, PGK1), fat metabolism (eg: FABP1), and the oxidative status (eg: GA3PDH). Furthermore, proteins linked to the onset of non-communicable diseases, including Type-2 diabetes and cardiovascular diseases (eg: APOA-1, and GA3PDH), were observed in FL mink. Low protein provision after weaning induced a significantly ($P < 0.05$) higher hepatic fat content in LP kits compared to controls and an age dependent change in FABP1 occurred in the proteome ($P < 0.005$). In conclusion, this study demonstrates a clear effect of fetal life protein restriction on the abundance of proteins involved in key metabolic processes and the potential development of non-communicable disease in adult life, and identifies a number of proteins that may be of interest for future mink research.

GENETIC AND MANAGEMENT SESSION

Development of the body condition score of mink during the implantation and gestation period is correlated with the whelping results

J. de Rond

Edelveen Research Farm has developed feed intake curves for mink in 2009 and 2010. In these curves the feed supply is related to the body condition of the female mink. The body condition is expressed as the Body Score (BS) and measured in steps of 0.5, ranging from BS 1.5 – BS 3.5. The application of the feed intake curves was tested from 2011 to 2013 by determining the BS after the last mating, after the implantation period and at the end of the gestation period. The curves were intended to result in a mild increase in BS from mating until the end of gestation. The number of live born kits per mated female of 2865 young females (YF) and 1904 old females (OF) (both minimal 2 times mated) between 2009 and 2013 is related to the BS development both during the implantation period and during the gestation period.

During the implantation period (2009 to 2013) 6% of the females had a decrease in BS, 66% remained at the same BS and 28% showed an increase in BS.

During the gestation period 5% of the females had a negative BS development, 60% no growth and 35% showed a growth in BS. The BS development during the implantation period had no relation with the whelping result, but among the females with 1 BS growth there was a higher incidence of empty females compared to those with no growth (5% versus 12 %). In relation with the gestation period however the number of live born kits is significantly lower for females with a negative BS growth in both YF (5.2 vs 6.2) and OF (6.5 vs 7.0) compared to no growth. Young females with growth in the gestation period have significant more live born kits compared to animals with no growth (6.5 vs 6.2). On average the highest number of live born kits were found in females with an increase of 1BS during the gestation period (YF 6.8 - OF 7.5 $p < 0,1$).

Feeding the mink what results in no BS growth during implantation and moderate BS growth during gestation will lead to the highest number of live kits born.

Maternal effect of heterosis revealed from analyses of crosses between mink lines

J.P. Thirstrup, P.F. Larsen, C. Pertoldi, J. Jensen

In the present study we analyzed crosses between different mink lines in order to estimate heterosis and variance components for litter size. We analyzed data from three Danish mink farms with different breeding strategies, which allowed for analyses of various crossing combinations.

Heterosis as well as variance components were estimated using the DMU package for analyzing multivariate mixed models. Effects of parity and production year, line origin and heterozygosity for female and offspring, as well as effect of permanent environment (due to repeated litters from the same female) and dam of the female (due to sisters selected for breeding) were included in the analyses. We found significant general heterosis for litter size for females and analyses of specific heterosis showed significant positive effect of crossing between lines within the same color type. Estimates of variance components revealed that heritabilities were 0.15, 0.06 and 0.09 for farm A, B, and C. Effects of permanent environment were 0.005, 0.11, and 0.15, respectively.

Feed efficiency and body weight growth throughout growing-furring period in mink using random regression method

M. Shirali, V.H. Nielsen, S.H. Møller, J. Jensen

The aim of this study was to determine genetic background of longitudinal residual feed intake (RFI) and body weight (BW) growth in farmed mink using random regression methods considering heterogeneous residual variances. Eight BW measures for each mink was recorded every three weeks from 63 to 210 days of age for 2139 male mink and the same number of females. Cumulative feed intake was calculated six times with three weeks interval based on daily feed consumption between weighing's from 105 to 210 days of age. Heritability estimates for RFI increased by age from 0.18 (0.03, standard deviation (SD)) at 105 days of age to 0.49 (0.03, SD) and 0.46 (0.03, SD) at 210 days of age in male and female, respectively. The heritability estimates for BW growth increased with age and had moderate to high range for male (0.33 ± 0.02 to 0.84 ± 0.02) and female (0.35 ± 0.03 to 0.85 ± 0.02). RFI estimates at the growing period showed large positive genetic correlations with the pelting RFI (210 days of age) in male (0.86 to 0.97) and female (0.92 to 0.98). Furthermore, BW records in the growing period had moderate (male: 0.39, female: 0.45) to large (male: 0.84, female: 0.91) genetic correlations for BW estimates at the growing period with the pelting BW (210 days of age). The result of current study showed that RFI and BW in mink are highly heritable, suggesting potential for large genetic gain for these traits, especially at the late furring period. The genetic correlations suggested that substantial genetic gain can be obtained by only considering RFI estimate and BW at pelting, however, lower genetic correlations than unity indicate that extra genetic gain can be obtained by including estimates of these traits at the growing period. This study suggests random regression methods are suitable for analysing feed efficiency and BW growth; and genetic selection for RFI in mink is promising.

Genomic selection in mink (*Neovison vison*): A simulation study

K. Meier, A.C. Sørensen, J.P. Thirstrup, M.S. Lund

The aim of this study was to evaluate the use of genomic selection in Mink breeding by means of stochastic simulations. Currently, breeding value estimation based on pedigree and phenotypic records is possible for the individual farmer. Traits implemented in the breeding goal differ in complexity, and so current breeding progress differs between individual traits. By comparing the current breeding schemes with different scenarios of genomic selection based on different accuracies and genotyping strategies, we assessed the genetic gain and total economic gain for the traits analysed.

Our results show that using genomic selection will improve total economic gain compared to traditional breeding. The total economic gain varies dependent on the accuracy and genotyping strategy, but even with low accuracy and low genotyping effort (10% of the males), total economic gain is higher compared to traditional breeding. Finally, our results show that it is possible to increase genetic gain for litter size, pelt quality and pregnancy rate, traits that have so far been difficult to improve with traditional breeding.

The next step will be to 1) conduct a sensitivity analysis to assess the impact of different economic weights, and 2) make a cost-benefit analysis to assess the genotyping cost relative to the total genetic merit. On a larger scale, the evaluation of genomic selection will involve an assessment of a potential future infrastructure supporting the method along with genotyping to assess the accuracy with genomic selection in mink.

Genetic and phenotypic correlations between fur quality traits and size evaluated on live animals and skin

J. Thirstrup, J. Jensen, K. Meier, A.C. Sørensen, M.S. Lund

The aim of the study was to evaluate how appropriate it is to select animals for breeding based on fur quality traits and size evaluated on live animals. To answer this question, we estimated heritabilities as well as genetic and phenotypic correlations between fur quality traits evaluated on live animals and on skin. For the analyses, we used

multivariate mixed models and performed the analyses in DMU. Bodyweight was negatively correlated to fur quality traits evaluated on skin whereas quality, guard hair thickness and silky evaluated on live animals were the single traits most highly and positively correlated to fur quality traits evaluated on skin. Due to correlations between fur quality traits evaluated on live animals and skin are far from 1 and due to negative correlation between bodyweight and other fur quality traits we recommend a) a breeding scheme in which traits subject for selection can be weighted according to the aim of the breeding goals b) selection based on fur quality evaluated on skin; c) genomic selection, or, at best, a combination of all three.

VETERINARY AND PATHOLOGY SESSION

Rationalization of the antimicrobial use in the Danish mink (*Neovison vison*) production using pharmacokinetics

T. Struve, L. Persson, E. Sjoblom, H. L. Frandsen, T. Clausen, R. Dam Tuxen, A.S. Hammer, M. Kreilgaard

During the past 10 years the antimicrobial administration in fur producing animals has been increasing dramatically. Currently there are no antimicrobial agents approved for treatment of infections in mink. Therefore the dosage interval, amount of compound and route of administration are based on an empirical assessment from the practicing veterinarian. Mink differs widely from the other production animals by its carnivorous anatomy and physiology by having a very short gastrointestinal tract and a relatively fast passage time. This might pose a risk of not only ineffective antimicrobial treatment but also a risk of contributing to development of antimicrobial resistance. In this study, one of the most commonly used antimicrobial agents (amoxicillin/clavulanic acid) were investigated by administering a single dose of the antimicrobial agent to clinically normal mink (n= 12) orally (n=6) or intramuscular (n=6). Serial plasma samples were collected over 24 hours, and antimicrobial concentration was determined using a High performance liquid chromatography-mass spectrometry assay. Using plasma concentration versus time, a non-compartmental pharmacokinetic analysis was performed. The

resulting C_{max} , V_d , T_{max} and AUC, CL, F and $t_{1/2}$ were used to compare the therapeutic effect of the antimicrobial agent and different administration routes.

The health situation in Norwegian fur farms 2012-2014

C.K. Heimberg

To get an overview over and improve the health situation in Norwegian fur animals, the Norwegian Fur Breeding Association introduced a mandatory health service. During the veterinary visits questionnaires for every period are filled out and handed in to the association. The prevalence of hereditary disorders in foxes in February and illnesses and injuries in mink in May as well as for all fur animals in June/July and October/November are evaluated over three years. This applies for reproduction performance and mortality rates also. The reproduction performance in silver foxes and mink is rather stable, but decreasing in blue foxes. The prevalence of disorders, injuries and mortality rates in Norwegian fur animals is generally low. There are big variations in different findings between the farms.

Canine distemper virus in free ranging terrestrial carnivores in Denmark and the association with high numbers of distemper outbreaks on mink farms

L.S. Albrechtsen, A. Jacobsen, L. Andresen, L. Nielsen, C.R. Olesen, J.F. Agger, S. Bildt, M.W.G. Van de Bildt, T. Kuiken, A.S. Hammer

Canine distemper virus (CDV) is one of the most important causes of infectious disease in wildlife populations affecting a wide variety of species across the world. It can also cause severe disease in domestic dogs and in the fur animal production. In 2012 an unusually high number of CDV outbreaks occurred on Danish mink farms in Jutland. The reasons for this sudden increase in outbreaks could be linked to a possible reservoir of the virus in wild carnivores.

In 2013 a project was initiated at Copenhagen University with the aim to assess the prevalence of CDV in free-ranging terrestrial carnivores in Denmark based on active sample collection and

laboratory analysis by real-time reverse transcription polymerase chain reaction (RT-PCR). Moreover, the possible linkage between disease occurrence in Danish wildlife and the numerous outbreaks of CDV in mink farms was evaluated by sequence and phylogenetic analysis. Lastly, a case-control designed questionnaire survey was conducted in collaboration with Copenhagen Fur to gain a better insight of the biosecurity measures used in fur animal production.

A total of 266 animals were tested for CDV, including the species red fox (*Vulpes vulpes*), Eurasian badger (*Meles meles*), beech marten (*Martes foina*), and free-ranging American mink (*Neovison vison*). The animals originated mostly from the peninsula of Jutland, but also from the islands of Fyn, Zealand and Bornholm. Seven foxes from Jutland were tested positive for CDV resulting in a regional prevalence in southern Jutland of 12.2% and 1.3% in central Jutland. Sequence analysis of the phosphoprotein gene region showed a high degree of similarity between the CDV isolates from the red foxes and an isolate from a CDV-positive farmed mink taken during the outbreaks of 2012. Furthermore, the field isolates were very similar to German strains and other European isolates.

The questionnaire based biosecurity survey was a matched case-control study conducted in both CDV-free farms and farms tested CDV-positive during the distemper outbreaks of 2012 and early 2013. The results revealed a great variation in the biosecurity procedures in use on Danish mink farms with a slight tendency of farms without CDV to have a higher level of biosecurity in place. Farm contact with wild foxes was associated with having experienced an outbreak of distemper. A higher number of correct and thorough vaccination strategies were connected with not having experienced an outbreak of CDV in 2012/2013.

In conclusion, the results of this study have shown CDV to be present in Danish red foxes with the highest prevalence in southern Jutland and strongly suggest that CDV has travelled from Germany to Denmark. Furthermore, the results indicate that the disease has spread from wild foxes to farmed mink. The general shortage of consistent use of biosecurity measures, proper vaccination strategies and the high degree of human traffic in and out of the mink farms are important issues to address in order to prevent future outbreaks. It is also central to recognize the possible implications of CDV for wildlife

populations in Denmark and to acknowledge the risk of having a CDV reservoir present in Danish wildlife. Contamination of immunologically naïve domestic animals with CDV by free-ranging carnivores is now an actual risk factor.

Gross pathological and virological investigations of Danish mink kits with diarrhea

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

In a larger study at University of Copenhagen during 2013, 208 mink kits from farms with outbreaks of diarrhea were subject to full necropsies and fecal or colon samples were analyzed with qPCR (astro- and coronavirus) in order to reach further understanding of diarrhea in the pre- and post weaning period. The majority of minks submitted in the pre-weaning period (105 out of 109) had lesions consistent with pre-weaning diarrhea (sticky kits) and in these mink a high prevalence of astrovirus was detected. In the post-weaning minks 36 out of 68 had macroscopic signs of gastroenteritis. Few tested positive for astrovirus and none for coronavirus. The lack of macroscopic gastrointestinal signs and presence of pathologic lesions in other organs (such as the urinary tract and fatty liver syndromes) could indicate the involvement of other underlying diseases on some of the farms, causing unthriftiness and diarrhea. The finding of astrovirus in relation to pre-weaning diarrhea support earlier studies suggesting astrovirus as a causal factor, however other factors cannot be ruled out. Further studies are necessary in order to evaluate the involvement of coronavirus.

Necrotic Pyoderma in Fur Animals

H. Nordgren

A new, severe suppurative skin inflammation (pyoderma) was detected in Finnish fur animals in 2005. The disease seems to spread between farms especially when new animals are introduced to a farm. On the farm the disease spreads between animals causing severe and even fatal symptoms. It compromises animal welfare and causes considerable economic losses to the farmers.

The disease affects all fur animal species farmed in Finland: mink (*Neovison vison*), foxes (*Vulpes lagopus*) and finnraccoons (*Nyctereutes procyonoides*, a raccoon dog bred in Finland for fur industry). Pyoderma is the common feature in all of the species although the disease pattern differs slightly between the three species. In mink the lesions are in feet and facial skin as necrotic dermatitis, and in finnraccoons as painful abscesses between toes. In foxes the disease manifests as a severe conjunctivitis, which spreads rapidly to dermatitis of eyelids and facial skin. Similar signs have been documented earlier in farmed mink in Canada in 1996 and also in The USA in 1970. In Canada the onset of the disease was simultaneous with the beginning of the use of seal byproducts as a raw material in mink feed (1). To our knowledge signs seen in foxes and finnraccoons have not been described previously (3) A similar disease pattern has been observed in fur animals in Denmark, Poland, Netherlands, Spain, Iceland and most recently Sweden and Norway (personal communication from veterinarians treating fur animals).

A case-control study was performed in Finland in 2010-2011 to describe the entity and to identify the causative agent. The project included macroscopic and histological examination as well as microbiological examination of 99 fur animals, both diseased animals and healthy controls. The data confirmed the disease pattern seen on the field: minks have necrotic dermatitis in feet and the facial skin, finnraccoons painful abscesses in the paws and foxes severe conjunctivitis and purulent inflammation of eyelids and the facial skin. A common finding in all species in pathological examination was necrotic pyoderma. Histological examination revealed a severe chronic necrotic pyoderma with ulceration and crusting. Bacteriological studies revealed the presence of many potential causative bacteria, some of them being the same or closely related to bacteria causing suppurative skin lesions in marine mammals. These findings are highly interesting when considering the connection seen in Canada with outcome of the disease and using the seal byproducts as a mink feed. All these results are to be published later (3).

Bacterial etiology of diarrhea in mink kits

A.S. Hammer, L.B. Rasmussen, S. Hansen, L. Krarup, P.P. Damgaard, B. Aalbek

Outbreaks of diarrhea in mink kits during the pre-weaning and growth period are one of the most important factors associated with use of antimicrobial use in Danish fur production. For other production animals it has been possible to significantly reduce the use of antimicrobials in the modern production systems and targeted research efforts aimed at identification and control of the most important infectious organisms has been an important facilitator of this process. In farm mink there is limited knowledge concerning the role of bacterial organisms as causal factors. Potentially pathogenic bacterial organisms like *Escherichia coli* (Jørgensen et al. 1996, Vulfson et al 2001) and *Campylobacter* (Bell et al. 1990, Hammer et al 2005) are commonly found in outbreaks of diarrhoea in mink during the production season. Hemolytic *staphylococci* have also been associated with diarrhea in mink (Sledge et al. 2010). Since these bacteria are found on farms with outbreaks of diarrhea - as well as in healthy animals on farms without disease problems (Vulfson et al. 2001, Vulfson et al 2003, Hammer et al. 2005, Guardabassi et al. 2010) - their role as a primary causal organism remains unclear. This abstract represent a small part of a larger multidisciplinary project concerning causal factors of diarrhea in mink. This abstract only includes results concerning readily culturable bacterial organisms isolated from fecal samples.

In 2013 rectal swabs were collected from 150 farm mink submitted from 30 farm outbreaks of diarrhea - and in 2014 rectal swaps were collected from 196 healthy mink on a farm without disease problems. Submissions from outbreaks of diarrhea typically included 4-5 mink euthanised or found dead on the farms during the outbreak. The samples from healthy mink were sampled at pelting in the month of March. The collected swabs were subjected to bacteriological investigation.

Several different bacteria types were found in samples from mink submitted from outbreaks of diarrhea including: *Clostridium perfringens*, *Enterococcus durans*, *Enterococcus faecalis*, *Enterococcus faecius*, *Enterococcus hirae*, hemolytic and non-hemolytic *Escherichia coli*, *Lactobacillus sakei*, *Lactococcus lactis*, Nonhæmolytiske *Escherichia coli*, *Plesiomonas*

shigelloides, *Proteus mirabilis*, *Staphylococcus pseudintermedius*, *Streptococcus canis* and *Streptococcus gallolyticus*

Hemolytic *E.coli* were identified as the predominant bacterial type in 14 submissions from farms with outbreaks of diarrhea (potential pathogenic bacteria were considered predominant in several or all samples from same submission). In 4 submissions *Streptococci canis* were considered the predominant bacterial type, in 3 cases non-hemolytic *E.coli* bacteria were considered the predominant type and in 2 cases *Plesiomonas spp.* were considered the predominant type. In the remaining submissions plates were overgrown with proteus and could not be evaluated (7)- or a unspecific mixed bacterial flora were detected (3 submissions).

Hemolytic and non-hæmolytic isolates were isolated from both healthy animals and animals with diarrhea. Hemolytic *E.coli* was identified as a predominant bacterial type in a large proportion of samples from healthy animals. Significant differences were detected in comparison of isolated hemolytic *E.coli* in the feces from two groups of healthy animals, given different types of feed. Considerable variations in susceptibility were found among farms with outbreaks of diarrhea. Similar variable antimicrobial resistance in coli isolates from different mink farms, has been shown in previous investigations (Vulfson et al 2001).

Investigations of some of the hemolytic coli isolates were included as part of a veterinary master project in 2014. This master study included a total of 196 fecal samples derived from healthy mink and 54 samples derived from mink with diarrhea. The study included Pulsed Field Gel-electrophoresis (PFGE) typing of hemolytic coli isolates. The hemolytic coli isolates were identified as *E. coli* using MALDI-TOF-MS and selected isolates were investigated for internal relations using PFGE. Different genotypes were detected from the outbreaks of disease in both healthy mink and isolates from mink with diarrhea. Fewer genotypes were however detected from outbreaks of diarrhea when compared to the feces samples from healthy animals.

In this study we applied pathological, microbiological and molecular biological methods in the investigations of bacterial organisms commonly associated with diarrhea in mink kits. The presented study has provided more information of the infectious agents associated with diarrhoea in mink kits – as well as providing evaluation of tools for future epidemiological surveys and experimental model studies, which may facilitate future

investigations of causal factors of diarrhea in mink kits.

The results of this study indicate that some strains are more likely to be associated with diarrhea than others. Further studies including more samples from more farms will be analysed in order to identify and characterize such strains. It is possible that other factors (eg. virus) influence the susceptibility of the mink to the bacteria. This may be the case with *E.coli* lung infections seen during outbreaks of influenza in mink (Yoon et al. 2010). One considerable drawback of previous investigations may be that they have targeted single or few organisms eg. virus, coli or campylobacter - not recognizing interaction or synergies between several factors. Future research efforts may benefit from applying a more holistic and interdisciplinary approach to investigations of causal factors of diarrhoea in order to evaluate the interaction of factors.

What is diarrhea in fur animals all about?

J. Korpela

Diseases in the digestive tract are a common problem in fur animal farming during the intense weaning period in the autumn. They are expensive and labor intense for the farmer and for the whole fur animal industry, increases use of antibiotics and are a welfare problem for the fur animals. In addition some of the pathogens are zoonotic, and hence present a health hazard for the farmer and employees on the farm. The symptoms vary a lot between farms, and it seems that no common factor has been found to explain the problems.

During the years many pathogens has been isolated in autopsy and analysis of fecal samples from minks, foxes and Finn raccoon, but the more precise meaning of the microorganisms is not known. Symptoms look very much alike every year; still the possible pathogens found may differ from each other. Many possible reasons of enteric disease have been seen during the years.

What have we encountered during the years?

Cases of Campylobacter

Campylobacter (mainly *C. jejuni* and *C. coli*) was considered a big problem in the years 2007 – 2009. In almost all cases of enteric disease campylobacter was isolated. At first it was thought to be a

secondary finding without consequences for the animals. The symptoms were mainly seen in mink, manure being yellowish and foamy, poor growth despite good appetite and poor fur quality. Also some farmers and employees on farms got campylobacter-infection.

When more and more farms and individual animals were affected, FFBA initiated Campylobacter-project in 2009 to meet the demands of the farmers. Prevalence of campylobacter was determined in samples taken both on farm level and in the feed chain (i.e. poultry abattoir and feed kitchen). This was because of suspicion that poultry offal was the source of infection. In the project fecal samples, feed samples, raw material samples (poultry offal) and environmental samples were collected on farms, and analyzed for Campylobacter. Campylobacter could be found on farm level, but not in raw materials or feed. The role of Campylobacter in enteric disease still remained unclear. On farm level cases of enteric disease where campylobacter has been isolated is treated with tetracycline or penicillin. In many cases reinfection occurred, possibly due to lack of sanitary actions in attempts to control disease.

Cases of Lawsonia

Proliferative enteropathy has been reported in many different animal species, including blue fox. During summer and autumn 2011 and 2012 Lawsonia intracellularis was detected in several enteritis cases in blue foxes. The symptoms detected were diarrhea, poor growth, dry fur coat, fluid loss and rectal prolapses. Mortality was usually low. However, in some farms acute, hemorrhagic diarrhea with high mortality was seen. The affected animals were usually young animals. *L. intracellularis* was detected on histopathological examination and by PCR, and was in most of the cases the only pathogen found. In some cases coccidia, parvovirus, Campylobacter jejuni or bacteria belonging to the genus clostridium were also detected. Foxes were treated with tylosinfosfate (Tylan) and seemed to recover quickly. However, some relapses after treatment was also seen.

The source of Lawsonia intracellularis in the fox farms remained unclear. In a case in 1990 the source of infection was untreated pigs bowels, and it is possible that intestines of infected swine can be the source. It is not known how effectively the current preservation methods are to destroy Lawsonia intracellularis.

Cases of parvovirus

Parvovirus in Finn raccoon is known to cause diarrhea, especially in growing animals. Therefore vaccination against parvovirus is common on Finn raccoon farms. Parvovirus outbreaks are now and then seen on farms that are not vaccinating their animals.

In foxes it is not proven that parvovirus causes enteric disease. Healthy foxes can show high levels of antibodies, indicating a recent infection, although no symptoms have been seen. On the other hand, in some cases of enteric disease on fox farms the only cause found seems to be parvovirus. In recent years parvovirus has been suspected to be the cause of both enteric disease and reproduction failure in foxes. Some farmers vaccinate their foxes, especially those having a mixed production of fox and Finn raccoon.

In Finland all farmers vaccinate their mink against parvovirus enteritis. Enteric disease in mink is most probably caused by other pathogens than parvovirus.

Cases of coccidia

Oocysts of coccidia have been thought to be a secondary finding in analysis of fecal samples and in autopsy. During the last years massive occurrence of coccidia in fecal samples from mink and fox has been found. In mink it is usually *Isospora* or *Eimeria*, in foxes usually *Isospora*. Symptoms of enteric disease have also been present at farm level; diarrhea, poor growth and susceptibility to other pathogens causing enteric disease. For some reason the Finn raccoon seems not to be affected as much as the other farmed fur animal species.

Animals have been treated with toltrazuril (Baycox/Tratol/Toltarox), and recovery has been seen of some of the farms. However, due to damage in the intestines and impaired absorption a total recovery is not achieved. Animals remain smaller and fur quality poorer than for the unaffected animals on the farm.

Because of simultaneous bacterial infections (i.e. *Campylobacter*) a treatment with antibiotics has also been necessary for recovery.

A total decontamination of coccidia is impossible, but improved sanitary actions and preventive medication decreases infection pressure.

Success of treatments

Sometimes the treatment is successful, and sometimes symptoms reoccur immediately when treatment is ended. In some cases many different

antibiotic treatments are used during the weaning period. In many cases there is a failure in farming routines, and the diarrhea continues or reoccurs even if treatment was accurate.

The annually occurring "autumn diarrhea" is clearly not caused by a single pathogen, but is probably a combination of many possible pathogens. Farm management and hygiene is also playing a huge role in the occurrence of enteric disease. When trying to resolve the problems, it has become apparent that there also is a lack of research results in enteric diseases

Potential pathogens can be identified from the sample material, but the more precise meaning of the results is not known. There might also be potential pathogens, especially viruses, which are not diagnosed by methods used in conventional diagnostics of fur animal enteric diseases. The lack of knowledge can lead to unnecessary use of antibiotics and to false diagnoses.

What should we do about it?

To be able to resolve these issues a project has been initiated. The aim is to clarify the role of pathogens thought to be important, and which are found in the gut flora or feces. Also the development of diagnostic tools is included in the project. By better knowledge we hope to reduce and clarify use of antibiotics, and to develop diagnostic tools and good practices on farm level to avoid these problems.

The study has been started in spring 2014 and is planned to continue until the end of year 2015. The project is done in Evira (Finnish Food Safety Authority) in cooperation with FFBA (Finnish Fur Breeders' Association) and research partners from Denmark; University of Copenhagen (Section for Veterinary Pathology) and DTU (Technical University of Denmark, National Veterinary Institute). The project is funded by Makera (Maaseudun Kehittämisrahasto, Ministry of Agriculture and Forestry) and FFBA.

The aim is to clarify the role of pathogens thought to be important, and which are found in the gut flora or feces.

Does the discovery of a novel Amdovirus effect AMDV diagnostics?

K. Aaltonen, T. Sironen, A. Knuutila, O. Vapalahti

Aleutian disease is an immune-complex disease with mild to severe and even lethal outcome for the

mink. It causes significant losses to farmers by reducing the quality of the pelts and number of pups being born. A program to reduce the amount of infections and spreading of the disease has been in operation with variable results. This program has depended on the serological screening of animals prior to moving from farm to farm and similar serological monitoring of the status of disease free farms. Several PCR based methods have been developed to confirm results in cases where serological testing alone could not give definite answers.

The Aleutian disease is caused by the Carnivore amdoparvovirus 1 (previously Aleutian mink disease virus), a parvovirus belonging to the genus Amdovirus. This virus was also the only member of the genus, until in 2011 a novel amdovirus, Carnivore amdoparvovirus 2 (previously Gray Fox virus) was discovered by Li et.al.

A PCR assay based on the NS1 region was described by Jensen et.al. in 2011. We modified this PCR for use in the confirmatory diagnostic testing done in combination with the ELISA test, which is currently in use in Finland. The primers were used in a qPCR platform with SYBR Green chemistry. Standard dilution series was done, and previous positive and negative samples tested on performance evaluation.

The discovery of the novel amdovirus from the American foxes posed a question whether this virus or other yet unidentified amdoviruses could be present in the animal populations. It is not known whether this new virus is able to infect or cause symptoms cross-species. This leaves open the possibility of cross-reactivity in the serological tests. Sequence analysis revealed that the primers currently used could not detect the novel amdovirus. A new set of primers based in the NS1 region was designed to encompass all published sequences including the Carnivore amdoparvovirus 2. The two primer sets have been run in parallel to ensure detection of amdoviruses and possible separation of Carnivore amdoparvovirus 1 and 2.

Sequencing of plasmacytosis virus

E.E. Hagberg, U. Fahnøe, T. Struve, A.G. Pedersen

Plasmacytosis, also called Aleutian Disease (AD), is worldwide the most important disease in mink production. It is caused by Aleutian Disease Virus (ADV), a linear single-stranded DNA-virus

belonging to the family *parvoviridae* (DeCaro et al. 2012), which upon infection results in excessive deposition of immune complexes severely affecting animal health and welfare. In addition, fur quality and fertility is reduced, imposing financial loss to the farmers. There is no efficient cure or vaccine against AD, and therefore a national control program was implemented in Denmark in 1976 (Chriel, M. 2000). Danish law regulates this program, which has the aim to eradicate AD by identifying infected farms and preventing spread of infection e.g. by stamping out.

Despite these negative effects on animals and the industry, routes of transmission of ADV are poorly understood. Little is known about the viral diversity since previous molecular biological studies have focused on smaller regions of the genome, and only a handful of viral isolates have been sequenced (Li et al. 2012, Christensen et al. 2011, Farid et al. 2010, Gottschalck et al. 1991).

A better understanding of the complete ADV genome and its diversity, will make it possible to differentiate strains and thereby identify isolates during outbreaks, and thus examine viral transmission-routes. Therefore the aim of this study is to sequence the ADV genome using next generation sequencing (NGS).

Plantar and Palmar Foot Lesions in Farmed Mink

A. Jespersen, A.S. Hammer, H.E. Jensen, N. Bonde-Jensen, M.M. Lassus, J.F. Agger

Through recent years, the occurrence of outbreak-like episodes of foot lesions in farmed mink in several countries has increased the awareness on skin diseases affecting the feet of mink. The outbreak-like lesions have been described under the term pododermatitis or simply as ulcerative lesions, for which a cause is being sought. The occurrence of calluses on the feet of mink has been mentioned only briefly in the literature; yet, studies of paws from mink submitted to the Department of Veterinary Disease Biology, University of Copenhagen have revealed a large proportion of callus-like lesions in the plantar metatarsal area. The aims of the present study were to elucidate the occurrence of foot lesions in Danish farmed mink, looking into the pathology of lesions, and to compare findings of farmed mink with mink living in the wild.

POSTER SESSION**Large potential for acidification of mink slurry**

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

In Denmark, the fur farmers have to reduce the ammonia emissions from open housing systems. Kopenhagen Fur, Hyldgaard Staldservice and Agro Tech, has in collaboration, conducted a survey of operational and environmental effects of acidification of mink manure. This was conducted with the installation of a system that allows the collection and acidification of the slurry production in mink housing.

No effect of acidification on the water intake or urine pH in mink

M. Engbæk, L. Tinggaard, K.H. Meldgaard, P.F. Larsen

In recent years some mink breeders have reported good experiences with acidifying drinking water on their farm. Acidified drinking water is supposed to reduce urinary pH, improve the taste, and thereby increase the water intake.

Whether mink have a preference in relation to the acidity of drinking water was investigated. It was also tested if the acidified drinking water had an effect on urinary pH and water intake.

The digestibility of industrial fish during degradation

M. Engbæk, L. Tinggaard, K.H. Meldgaard, P.F. Larsen

The aim of the study was to investigate the digestibility of industrial fish degrading over time, and to compare the found digestibilities with the contents of TVN (Total Volatile Nitrogen) and biogenic amines.

Hemolytic *Escherichia coli* associated with pneumonia in farm mink (*Neovison vison*)

L.B. Rasmussen, P.P. Damborg, A.S. Hammer, B. Aalbæk

Infection with *Escherichia coli* has previously been associated with different types of illness in mink including hemorrhagic pneumonia. The infections cause both decreased welfare and increased mortality, thus representing both an economic and health concern in the mink production. The reason why *E.coli* causes disease in mink has not yet been explained and only few studies have previously dealt with the problem.

This abstract presents results of a pilot project, with the aim of getting closer to the pathogenesis behind *E.coli* lung infections. This was done by evaluation of hemolysis, typing and pathologic and histopathologic investigations.

The study included 13 swaps from lungs of mink with hemorrhagic pneumonia. All isolates showed hemolysis on bloodagar and were identified as *E.coli* by means of MALDI-TOF-MS. Additionally the isolates were investigated for internal relations by pulsed-field gel electrophoresis and histological preparations were submitted to histopathological investigations.

PFGE-typing was performed on both the 13 lung isolates and *E. coli* isolates from 9 fecal samples from healthy mink. Different genotypes were detected in both animal groups. Significantly fewer genotypes were detected from the samples of hemorrhagic pneumonia when compared to the fecal samples of healthy animals. By histopathological investigation the pneumonia were determined as acute hemorrhagic pneumonia.

More research concerning the role of *E.coli* as a pathogen and methods of disease prevention associated with *E.coli* bacteria is needed.

This abstract present results from a veterinary master project, which were conducted as part of a continuous program for veterinary students interested in Fur Animal health and disease. The program is supported in corporation by University of Copenhagen and Kopenhagen fur.

Assessment of climate data (temperature and air humidity) on mink farms and association with kit mortality during the month of June

N. Bloksgaard, L. Jensen, A.S. Hammer, A. Jespersen, T. Clausen, J.F. Agger

Mortality and wounds in mink pups are regarded as multifactorial problems. Wounds cause a significant proportion of the total mortality in mink pups, during their growth period. Occurrence of mortality and wounds in juvenile mink is a welfare problem and an economic problem for the industry. Today's increased public attention on animal welfare dictates that more research on the subject is needed.

The main purpose of this observational study is to assess the effect of temperature on mortality, due to "wounds" and "other causes of death" in mink kits in June 2012 and June 2013. With no previous published articles and limited research on the subject, it is not possible to compare the results within the topic of mink, but similar studies have been done for pigs and poultry.

The study was conducted in cooperation with the Danish experimental farm (Farm West) owned by the Danish mink farmers. Three randomly selected housing sections were equipped with climate data loggers, which logged the temperature (Celsius) every hour. Due to almost identical mean temperatures and a low number of deaths in the three housing sections, the study classified the farm as one unit. All the dead mink were collected, and autopsied determine the cause of death. 24,801 mink kits were included in the study.

A statistical coherence was found between "other causes of death (%)" and the temperature for both years. However, the correlations were opposing for the two years. In 2012 "other causes of death (%)" decreased when the temperature increased but in 2013 "other causes of death (%)" increased when the temperature increased. A positive correlation was found between "wounds (%)" and "the daily mean temperature" in 2013.

Theory regarding the temperature's effect on mortality in mink kits is still not sufficient, but the study concluded that the mean temperature could account for 9.8% of the mortality due to other causes than wounds. This study excluded all other variables, but temperature. Therefore, more research concerning the effect of temperature and other variables is needed.

This abstract present results from a veterinary master project, which were conducted as part of a continuous program for veterinary students interested in Fur Animal health and disease. The program is supported in corporation by University of Copenhagen and Kopenhagen fur.

Semen analysis program for blue fox (*Alopex lagopus*) and silver fox (*Vulpes vulpes*)

J. Sten

The breeding result of foxes, especially the blue fox, has been decreasing during the last ten years. Still, a good whelping result is the fundament of profitable fox farming. The mating season is short and intense in foxes, and is very labor intense for the farmer. Most of the foxes are artificially inseminated, and methods has remained the same for the last 40 years. Evaluation of the semen is performed by the farmer himself. However, new techniques has been developed for other animal species, and the aim is to develop a method to evaluate blue and silver fox semen as well.

Evaluation of fox semen, both silver fox and blue fox, is currently done subjectively on most fox farms in accordance to artificial insemination in mating season. This is not a precise method, and variability between evaluations done by different persons is significant.

The problems associated with manual evaluation of fox semen are for example exhaustion of eyes during long working hours, microscopes of varying quality, which leads to difficulties in evaluation of motility, morphology and mobility of the spermatozoa. In addition thickness of semen specimen is varying in thickness on normal glass slides, leading to varying results in evaluation of semen density in ejaculate.

In 2011 a project, Pälskraft, was initiated to develop farming in Ostrobothnia, Finland. The aim of the project is to develop farming routines on farm level and to raise the image of fur farming. As a part of this project new technology, for helping the farmer achieving a better breeding result on the farm, was searched for. The Pälskraft project is owned by SÖP (Svenska Österbottens Pälsdjursodlarförening). And funded by SÖP, Vasek, Concordia, Dynamo and feed kitchens in the Ostrobothnia area (Nyko frys, Nä-Rö frys, Molpe frys, Terjärv frys and Torp frys). The duration of the Pälskraft-project is 2011 – 2014.

Sedation procedure of farm-raised mink during electrophysiological measurements

H.T. Korhonen, S. Cizinauskas, J. Jesernics, P. Eskeli

The mink (*Neovison vison*) is a small member of the family Mustelidae. It typically has an elongated body shape, short legs and sexual dimorphism. Due to high surface-to-mass ratio, the mink have to sustain higher resting metabolic rate than other animals of the same body size (Brown & Lasiewski, 1972; Korhonen et al., 1983). This sets special demands for its medical treatment in laboratory experiments. Sedation and anaesthesia are mainly used for haematological and clinical-chemical examination and short-term surgical procedures in the mink (Jepsen et al., 1981; Aenemo and Søli, 1992; Wamberg et al., 1996). Any documentation is available on sedation during electrophysiological evaluations in this species.

In our previous electrophysiological study (Korhonen et al., 2009) we used medetomidine for sedation in farmed fox (*Alopex lagopus*). This drug worked well providing ideal sedation for measurements of electroencephalography (EEG), electrocardiography (EEC), and respiration. Medetomidine has been successfully used to induce immobilization in blue foxes, too (Jalanka, 1990). In the present study, the aim was to apply medetomidine sedation for electrophysiological measurements in farmed mink before and during euthanasia. This paper is based on original publication of Korhonen (2014).

Explorativity and confidence of foxes in enriched environment

H.T. Korhonen, P. Eskeli, T. Lappi

Housing environment of farmed blue foxes (*Vulpes lagopus*) must be designed to fulfill behavioural needs of the species in question as much as possible. One potential way for fulfillment would be to offer various specific enrichments for their use. In the present study, traditional farm fox cage was furnished with versatile equipments such as wire-netting platform, top nestbox, chewing bone, straw, digging plate, cord, roof rope, and ice hockey. The aim was to find out how multi-enriched environment affect blue foxes' behavioural reactions, i.e. explorativity and confidence.

Explorative behaviour of foxes was evaluated by a ball test in which a floorball was placed in the cage. Contact to ball was recorded. If there was no contact within 10 seconds, the test was aborted. Capture test was made before weighting of animals. Foxes were caught with neck tongs. Capture reaction was classified as confident or fearful. The results showed that explorativity of foxes markedly increased when animals were transferred from home cage to enriched cages. Explorativity remained high during entire study period when foxes were kept in enriched enrichment. Furthermore, it remained also after transferring of animals back to home cages. Capture reaction of foxes was quite similar in home and enriched cages. The conclusion here is that enriched environment increases foxes' explorativity and, thus, enhances animal welfare.

Fur properties in blue foxes fed ad libitum and restricted Ca:P diets

H.T. Korhonen, P. Eskeli, T. Lappi, J. Sepponen

The present paper provides results from study which clarified effects of ad libitum and restricted Ca:P diets on fur properties in blue foxes. Treatment groups were: (1) restricted feeding (R 1), Ca:P ratio 1.5:1; (2) restricted feeding (R 2), Ca:P ratio 2.9:1; (3) restricted feeding Ca:P-ratio control level (R 3); (4) ad libitum feeding (AL 1), Ca:P ratio 1.5:1; (5) ad libitum feeding (AL 2), Ca:P-ratio 2.9:1; (6) ad libitum feeding (AL 3) Ca:P ratio control level. Animals were pelted (Nov 26) according to normal farming practice. Skin grading was performed by Turkistila Luova Oy, Kannus. Fur characteristics evaluated were fur mass, cover of hair and quality. The scale ranged from 1 (poorest) to 10 (best). Fur defects were also evaluated. Skins were weighed with a Mettler SM 15 balance, accuracy ± 10 g. Skin length was measured by using a tape measure, accuracy ± 1 cm. Fur properties were analysed using the following linear mixed model:

$$Y_{ij} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ij},$$

where μ was the general mean, α_i was the fixed effect of feeding intensity (R or AL), β_j was the fixed effect of Ca:P ratio (1.5:1, 2.9:1, control level) and $(\alpha\beta)_{ij}$ was the interaction between fixed effects. ε_{ij} was the residual error. Clear differences were found in fur properties between the experimental groups. Skin length and weight was greater ($P < 0.001$) in foxes fed *ad libitum* compared to restrictedly fed ones. Furthermore, fur mass and fur

quality were better ($P < 0.05$) in *ad libitum* fed animals. Ca:P levels did not affect skin weight, length or fur mass of animals. The cover of hair and fur quality was poorest in animals fed 2.9:1 diet.

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