



Introduction: Dr. Hugh Hildebrandt

Fur Commission USA and Joint Mink Research Committee Adviser

Things are starting to get back to a little closer to normal. Ranch quarantines have been released, mink and ranches are caring for the new kit crop. With things starting back up, research projects are again ramping up and reports are again getting finalized.

This newsletter has a diverse mix of articles from genetic studies to clinical and feed information. As always the full research articles are available in the library if you are interested in a more in-depth description of the study than the abstract or summary provides.

I know from talking to many of you, hopefully soon we will be able to get together as we all miss our friendships and industry partners.



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All studies are available in their entirety at www.FurResearch.org, a free repository for academic research addressing health, welfare, and disease issues affecting the husbandry of fur bearing animals. Originally launched in 2005 by Fur Commission USA as an archive of the work done by the first generation of leading fur animal scientists in North America, we now include more recent material from sources across the globe, to be used as reference tools for current and future researchers, veterinarians, and fur farmers throughout the world. Fur Commission USA does not receive any compensation for this service.

Dietary supplementation of *Ascophylum nodosum* improved kidney function of mink challenged with Aleutian mink disease virus

A. Hossain Farid and Nancy J. Smith

Abstract;

Background: Feed additives which can ease the negative effects of infection by the Aleutian mink disease virus (AMDV) are of interest to mink farmers. The effects of kelp meal (*Ascophylum nodosum*) supplementation on immune response, virus replication and blood parameters of mink inoculated with AMDV were assessed. AMDV-free black mink (n = 75) were intranasally inoculated with a local strain of AMDV and fed a commercial pellet supplemented with kelp meal at the rates of 1.5% or 0.75% of the feed or were kept as controls (no kelp) for 451 days. Blood was collected on days 0 (pre-inoculation), 31, 56, 99, 155, 366 and 451 post-inoculation (dpi).

Results: No significant difference was observed among the treatments for the proportion of animals positive for antibodies against the virus measured by the counter-immunoelectrophoresis (CIEP), viremia measured by PCR, antibody titer measured by quantitative ELISA, total serum protein measured by a refractometer or elevated levels of gamma globulin measured by iodine agglutination test at the sampling occasions. At the termination of the experiment on 451 dpi, there were no differences among treatments for antibody titer measured by CIEP, total serum protein, albumin, globulins, albumin:globulin ratio, alkaline phosphatase, gamma-glutamyl transferase, and proportions of PCR positive spleen, lymph node or bone marrow samples, but blood urea nitrogen and creatine levels were significantly lower in the 1.5% kelp supplemented group than in the controls.

Conclusion: Kelp supplementation improved kidney function of mink infected with AMDV with no effect on liver function, immune response to infection by AMDV or virus replication.

Keywords: Aleutian mink disease virus; American mink, *Ascophylum nodosum*, Kidney function, Serum profile

Effects of dietary kelp (*Ascophylum nodosum*) supplementation on survival rate and reproductive performance of mink challenged with Aleutian mink disease virus

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Abstract: Infection with Aleutian mink disease virus (AMDV) has negative effects on reproductive performance and survival rate of American mink (*Neovison vison*). The objectives of this study were to assess the effects of kelp (*Ascophyllum nodosum*) supplementation on survival, growth rate, and reproductive performance of mink challenged with AMDV. AMDV-free female black mink ($n = 75$) were intranasally inoculated with a local AMDV strain. Mink were fed a commercial pellet supplemented with 1.5% or 0.75% kelp or were kept as controls (received no kelp) for 451 d. Body weight and rectal temperature were recorded on days 0, 31, 56, 99, 155, 366, and 451 post inoculation (PI). Annual mortality rates were 13.6%, 20.0%, and 31.8% for mink fed 1.5%, 0.75%, or 0.0% kelp, respectively ($P = 0.29$). Mink which were fed 1.5% kelp had a significantly ($P < 0.01$) greater daily weight loss during breeding and post-breeding periods (days 155–366 PI), and outperformed ($P < 0.01$) the other groups in regard to litter sizes at birth and weaning. Differences among treatments were not significant for the number of females mated, or whelped of those exposed to males, kit survival from birth to weaning, or rectal temperature. It was concluded that 1.5% kelp supplementation had beneficial effects on survival rate of adult mink and litter size.

Key words: Aleutian mink disease virus, American mink, *Ascophyllum nodosum*, body weight, reproductive performance, survival rate.

Dose response of black American mink to Aleutian mink disease virus

A. Hossain Farid | Irshad Hussain

Abstract

Introduction: Aleutian mink disease virus (AMDV) causes a serious health problem for mink globally. The disease has no cure nor an effective vaccine and selection for tolerance using antibody titer is adopted by many mink farmers. The objective of this study was to investigate the effects of various doses of a local AMDV isolate on the response of black American mink to infection with AMDV.

Methods: Eight black American mink were each inoculated intranasally with 0.5 mL of eight serial 10-fold dilutions (100 to 10⁻⁷) of a 10% spleen homogenate containing a local AMDV isolate. Blood samples were collected on days 0, 20, 35, 56, 84, 140, and 196 post-inoculation (dpi). Anti-AMDV antibodies and viral DNA were tested by counter-immunoelectrophoresis (CIEP) and PCR, respectively. Animals that were PCR or CIEP positive at 196 dpi ($n = 41$) were killed at 218 dpi, and samples of blood and seven organs were tested by CIEP and PCR.

Results: Antibody production persisted in all seroconverted mink until the termination of

the experiment, whereas 71.1% of the mink showed short-lived viremia.

Significant associations were observed between inoculum dose and the incidence of viremia until 84 dpi which disappeared thereafter, whereas associations between inoculum dose and the incidence of seropositive mink were significant on all sampling occasions. Antibody titer at 218 dpi significantly decreased with decreasing inoculum dose. AMDV DNA was detected in the bone marrow, lymph nodes, and spleen samples of almost all mink inoculated at every dose but was not detected in other organs of some mink.

Conclusions: CIEP is more accurate than PCR for detecting AMDV infection in mink. Using antibody titer in naturally infected mink may not be accurate for the identification of tolerant mink.

KEYWORDS

Aleutian mink disease virus, American mink, anti-AMDV antibody, dose-response relationship, Viremia

Evaluation of Growth Curve Models for Body Weight in American Mink

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Simple Summary: Understanding the animal growth is important for optimized management and feeding practices as well as genetic improvement of animals; however, little is known about the growth of mink raised in Canada. This study evaluated the performances of ten models to find the best models describing the growth curves in mink. The results showed that Logistic and Richards were the best model for males and females, respectively. Growth curves were different between males and females. These results suggested that Richards model can be used for modelling the mink growth and modelling might be performed separately for male and female individuals.

Abstract: Modelling the growth curves of animals is important for optimizing the management and efficiency of animal production; however, little is known about the growth curves in American mink (*Neovison vison*). The study evaluated the performances of four three-parameter (Logistic, Gompertz, von Bertalanffy, and Brody), four four-parameter (Richards, Weibull, Bridges, and Janoscheck) and two polynomial models for describing the growth curves in mink. Body weights were collected from the third week of life to the week 31 in 738 black mink (373 males and 365 females). Models were fitted using the `nls` and `nlsLM` functions in `stats` and `minpack.lm` packages in R software, respectively.

The Akaike's information criterion (AIC) and Bayesian information criterion (BIC) were used for model comparison. Based on these criteria, Logistic and Richards were the best models for males and females, respectively. Four-parameter models had better performance compared to the other models except for Logistic model. The estimated maximum

weight and mature growth rate varied among the models and differed between males and females. The results indicated that males and females had different growth curves as males grew faster and reached to the maximum body weight later compared to females. Further studies on genetic parameters and selection response for growth curve parameters are required for development of selection programs based on the shape of growth curves in mink.

Keywords: body weight; growth curve; mink; non-linear models

Tail Tip Hair Loss in Mink a Case Report

Dale Miskimins DVM MS, Hugh Hildebrandt DVM

The goal of this study was to examine the potential causes of hair loss at the tip of the tail in a small population of mink on a ranch. This hair loss results in a smooth hairless tip with no visible signs of skin damage.

The study was set up to determine the cause. Determination if trauma to deeper tissues of the tail existed, some infectious issue either bacterial or parasitic causing irritation was present or if nerve damage to the spine or tail base from handling was a causative problem.

Two mink with smooth tail tips were submitted for a histological work up including decalcification of the spinal column.

Results showed no lesions in the brain, spinal cord, skeletal muscle or skin from the tail tip. No inflammation or trauma was observed on the skin of the two mink. The examination showed that the affected animals pulled the hair out, rather than chewed the hair out.

The results of this study would indicate that for this condition trauma, infection or aggressive handling were not concerns and the condition should be managed with genetic selection and environmental enrichments.

Opportunities for genomic selection in American mink: A simulation study

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Abstract; Genomic selection can be considered as an effective tool for developing breeding programs in American mink. However, the genetic gains for economically important traits can be influenced by the accuracy of genomic predictions. The objective of

this study was to investigate the prediction accuracies of traditional best linear unbiased prediction (BLUP), multi-step genomic BLUP (GBLUP) and single-step GBLUP (ssGBLUP) methods in American mink using simulated data with different levels of heritability, marker density, training set (TS) sizes and selection designs based on either phenotypic performance or estimated breeding values (EBVs). Under EBV selection design, the accuracy of BLUP predictions was increased by 38% and 44% for $h^2 = 0.10$, 27% and 29% for $h^2 = 0.20$, and 5.8% and 6% for $h^2 = 0.50$ using GBLUP and ssGBLUP methods, respectively. Under phenotypic selection design, the accuracies of prediction by ssGBLUP method were 11.8% and 15.4% higher than those obtained by GBLUP for heritability of 0.10 and 0.20, respectively. However, the efficiency of ssGBLUP and GBLUP was not influenced by selection design at higher level of heritability ($h^2 = 0.50$). Furthermore, higher selection intensity increased the bias of predictions in both pedigree-based and genomic evaluations. Regardless of selection design, TS sizes for GBLUP and ssGBLUP methods should be at least 3000 to achieve more accuracy than using BLUP for heritability of 0.50 and marker density of 10k and 50k. Overall, more accurate predictions were obtained using ssGBLUP method particularly for lowly heritable traits and low density of markers. Our results indicated that TS sizes should be optimized in accordance with heritability level, marker density, selection design and prediction method for genomic selection in American mink. The results provided an initial framework for designing genomic selection in mink breeding programs.

Detection of selection signatures for response to Aleutian mink disease virus infection in American mink

*Karim Karimil, A. Hossain Faridl, Sean Myles2 & Younes Miarl**

Aleutian disease (AD) is the most significant health issue for farmed American mink. The objective of this study was to identify the genomic regions subjected to selection for response to infection with Aleutian mink disease virus (AMDV) in American mink using genotyping by sequencing (GBS) data. A total of 225 black mink were inoculated with AMDV and genotyped using a GBS assay based on the sequencing of ApeKI-digested libraries. Five AD-characterized phenotypes were used to assign animals to pairwise groups. Signatures of selection were detected using integrated measurement of fixation index (FST) and nucleotide diversity ($\theta\pi$), that were validated by haplotype-based (hap-FLK) test. The total of 99 putatively selected regions harbouring 63 genes were detected in different groups.

The gene ontology revealed numerous genes related to immune response (e.g. TRAF3IP2, WDR7, SWAP70, CBF3, and GPR65), liver development (e.g. SULF2, SRSF5) and reproduction process (e.g. FBXO5, CatSper β , CATSPER4,

and IGF2R). The hapFLK test supported two strongly selected regions that contained five candidate genes related to immune response, virus–host interaction, reproduction and liver regeneration. This study provided the first map of putative selection signals of response to AMDV infection in American mink, bringing new insights into genomic regions controlling the AD phenotypes.

Linkage Disequilibrium, Effective Population Size and Genomic Inbreeding Rates in American Mink Using Genotyping-by-Sequencing Data

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Knowledge of linkage disequilibrium (LD) patterns is necessary to determine the minimum density of markers required for genomic studies and to infer historical changes as well as inbreeding events in the populations. In this study, we used genotyping-by-sequencing (GBS) approach to detect single nucleotide polymorphisms (SNPs) across American mink genome and further to estimate LD, effective population size (N_e), and inbreeding rates based on excess of homozygosity (FHOM) and runs of homozygosity (ROH). A GBS assay was constructed based on the sequencing of ApeKI-digested libraries from 285 American mink using Illumina HiSeq Sequencer. Data of 13,321 SNPs located on 46 scaffolds was used to perform LD analysis. The average LD (r^2 \pm SD) between adjacent SNPs was 0.30 ± 0.35 over all scaffolds with an average distance of 51 kb between markers. The average $r^2 < 0.2$ was observed at inter-marker distances of >40 kb, suggesting that at least 60,000 informative SNPs would be required for genomic selection in American mink. The N_e was estimated to be 116 at five generations ago. In addition, the most rapid decline of population size was observed between 100 and 200 generations ago. Our results showed that short extensions of homozygous genotypes (500 kb to 1 Mb) were abundant across the genome and accounted for 33% of all ROH identified. The average inbreeding coefficient based on ROH longer than 1 Mb was 0.132 ± 0.042 . The estimations of FHOM ranged from 0.44 to 0.34 among different samples with an average of 0.15 over all individuals. This study provided useful insights to determine the density of SNP panel providing enough statistical power and accuracy in genomic studies of American mink. Moreover, these results confirmed that GBS approach can be considered as a useful tool for genomic studies in American mink.

Keywords: American mink, genotyping-by-sequencing, single nucleotide polymorphism, linkage disequilibrium, effective population size, inbreeding rate

Selection for Favorable Health Traits: A Potential Approach to Cope with Diseases in Farm Animals

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Simple Summary: The losses caused by the outbreak of diseases are disastrous for the animal farming industries. There is an urgent need for an ancient, economical, and permanent disease control method to cope with the adverse effects of diseases in farm animals. In this review, we have proposed that genetic/genomic selection for animals with favorable health traits provide potential methods to eliminate the adverse influences of diseases in farm animals. It is undeniable that the traditional methods for disease control (e.g., vaccination, treatment, and eradication strategy) and several other rising disease control and detection methods (e.g., genome editing, biosensor, and probiotics) are contributing to the prevention of diseases from farm animals, curing infected animals, and detecting sick individuals; however, the limitations and deficiencies of these methods cannot be ignored. Although genetic/genomic selection solutions are facing some challenges, the developments of selection-associated techniques (e.g., high throughput phenotyping and sequencing, and generation of big data) and the advantages of selection over the other disease control methods can provide animal farming industries the ability to cope with the issues caused by diseases through breeding for health traits.

Abstract: Disease is a global problem for animal farming industries causing tremendous economic losses (>USD 220 billion over the last decade) and serious animal welfare issues. The limitations and deficiencies of current non-selection disease control methods (e.g., vaccination, treatment, eradication strategy, genome editing, and probiotics) make it difficult to effectively, economically, and permanently eliminate the adverse influences of disease in the farm animals. These limitations and deficiencies drive animal breeders to be more concerned and committed to dealing with health problems in farm animals by selecting animals with favorable health traits. Both genetic selection and genomic selection contribute to improving the health of farm animals by selecting certain health traits (e.g., disease tolerance, disease resistance, and immune response), although both of them face some challenges. The objective of this review was to comprehensively review the potential of selecting health traits in coping with issues caused by diseases in farm animals. Within this review, we highlighted that selecting health traits can be applied as a method of disease control to help animal agriculture industries to cope with the adverse influences caused by diseases in farm animals. Certainly, the genetic/genomic selection solution cannot solve all the disease problems in farm animals. Therefore, management, vaccination, culling, medical treatment, and other measures must accompany selection solution to reduce the adverse impact of farm animal diseases on profitability and animal welfare.

Keywords: diseases; farm animals; genetic selection; health traits

Research report: Fat liver syndrome in the mink—preliminary proof of dialogue between adipose tissue and liver through extracellular vesicles as a cause of hepatic lipid accumulation

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Summary; The research team at the University of Eastern Finland (UEF) has completed the studies allocated to the funding by the Mink Research Committee (Canada). The funding provided us with important resources to hire a PhD student and to purchase the necessary reagents and equipment to conduct the research. The main findings of the project were as follows:

The hypothesis of orotic acid as a suitable tool for the whole process of adipose transformation could not be verified. Instead, orotic acid only triggered the first hit of the fat liver model (Matilainen et al. 2020).

Instead, we have found evidence that adipose tissue signals the liver through extracellular vesicles causing the hepatocytes to become expressive of the adipose cell phenotype. This dialogue between adipose tissue and liver needs to be studied further as it provides a very promising tool to translational studies to treat this condition in domestic animals and human populations.

Research report: Fat liver syndrome in the mink—preliminary proof of dialogue between adipose tissue and liver through extracellular vesicles as a cause of hepatic lipid accumulation

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Abstract

Background: Orotic acid (OA) has been intensively utilized to induce fatty liver in rats. Although the capacity of OA to cause steatosis is species-specific, previous in vitro studies indicate that humans could also be susceptible to OA induced fatty liver. The aim of the present study was to re-elucidate the potential of OA exposure to modulate the cellular mechanisms involved in both non-alcoholic fatty liver disease pathogenesis and cellular

protection from lipid accumulation. In addition, alterations in detailed fatty acid (FA) profiles of cells and culture media were analyzed to assess the significance of lipid metabolism in these phenomena.

Methods: In our experiments, human hepatocellular carcinoma HepG2 cells were exposed to OA. Bacterial endotoxin, lipopolysaccharide (LPS), was used to mimic hepatic inflammation. The lipogenic and inflammatory effects of OA and/or LPS on cells were assessed by labeling cellular lipids with Nile red stain and by performing image quantifications. The expression levels of key enzymes involved in de novo lipogenesis (DNL) and of inflammatory markers related to the disease development were studied by qRT-PCR. FA profiles of cells and culture media were determined from total lipids with gas chromatography–mass spectrometry.

Results: Our data indicate that although OA possibly promotes the first stage of DNL, it does not cause a definite lipogenic transformation in HepG2 cells. Reduced proportions of 16:0, increased stearoyl-Coenzyme A desaturase 1 mRNA expression and relatively high proportions of 16:1n-7 suggest that active delta9-desaturation may limit lipogenesis and the accumulation of toxic 16:0. Inflammatory signaling could be reduced by the increased production of long-chain n-3 polyunsaturated FA (PUFA) and the active incorporation of certain FA, including 18:1n-9, into cells. In addition, increased proportions of 20:4n-6 and 22:6n-3, total PUFA and dimethyl acetal 18:0 suggest that OA exposure may cause increased secretion of lipoproteins and extracellular vesicles.

Conclusions: The present data suggest that, apart from the transcription-level events reported by previous studies, modifications of FA metabolism may also be involved in the prevention of OA-mediated steatosis. Increased delta9-desaturation and secretion of lipoproteins and extracellular vesicles could offer potential mechanisms for further studies to unravel how OA-treated cells alleviate lipidosis.

Keywords: De novo lipogenesis, Hepatocyte, Inflammation, Lipidosis, Non-alcoholic fatty liver disease

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