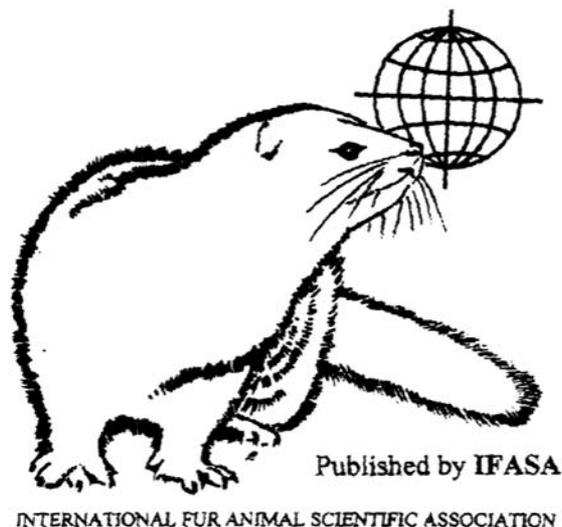


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## **Management of health in mink** ***A HACCP plan for energy allowance during winter and gestation in order to control sticky kits***

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### **Abstract**

Mink production is characterised by a strict annual production cycle in which all animals are naturally synchronous and the number of animals in a given mink production period is high. Each age group presents its own health problems and the whole herd is at risk to period-specific hazards. Due to a significant time lag in the feedback-loop of mink management, health management should focus strongly on preventive measures. The HACCP principles offer a systematic approach to the development of preventive measures aimed at annually recurrent health problems. The hazard of inadequate energy supply during the winter and during pregnancy has been identified as a risk factor for pre-weaning diarrhoea. The severity and seriousness of the consequences of this hazard has been analysed at farm level based on production data from 5 years (1994-1998) from a total of 125 farms in Denmark. Two hazards for pre-weaning diarrhoea at farm level have been identified: 1). Severely restricted feeding during the winter followed by a high energy allowance during the flushing period. A difference between the Flushing and Conditioning periods of 90 kcal/female/day at farm level increases the risk (OR = 3.03;  $p=0.01$ ). 2). A high energy allowance during the implantation period and the first part of the gestation period followed by a drastic decrease in the later part of the gestation period. By each kcal/female/day in difference between the Implantation and Prenatal two periods the risk of pre-weaning diarrhoea at farm level increases by OR=1,013 ( $p<0,05$ ). A difference between the two periods of 50 kcal/female/day at farm level increases the risk by OR = 1,91. Following the hazard evaluation the average feed allowance in kcal/female/day can be

identified as Critical Control Point for both hazards. Critical limits may be established as the average  $\pm$  one unit of STD for the group of farms without pre-weaning diarrhoea or  $\pm$  20 kcal relative to an estimated "need" of 210 kcal/female/day.

### **Introduction**

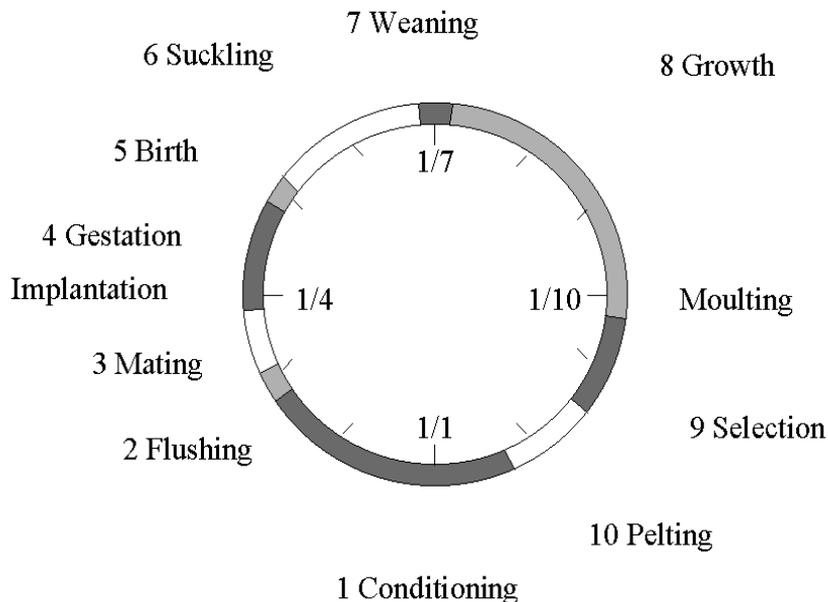
The general health status of the farm mink is good and the mortality is low apart from the first days after birth (Dietz et al., 2000; Durrant, 2000; Rattenborg et al. 1999; Schneider & Hunter 1993a; 1993b) while use of antimicrobials varies considerably between farms and production periods (Chriél & Dietz, 2000).

The management of health in mink production is to a large extent defined by the same factors that in general characterise management of synchronised production systems: a time lag in the management feed back cycle.

### *Seasonal synchrony*

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

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



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

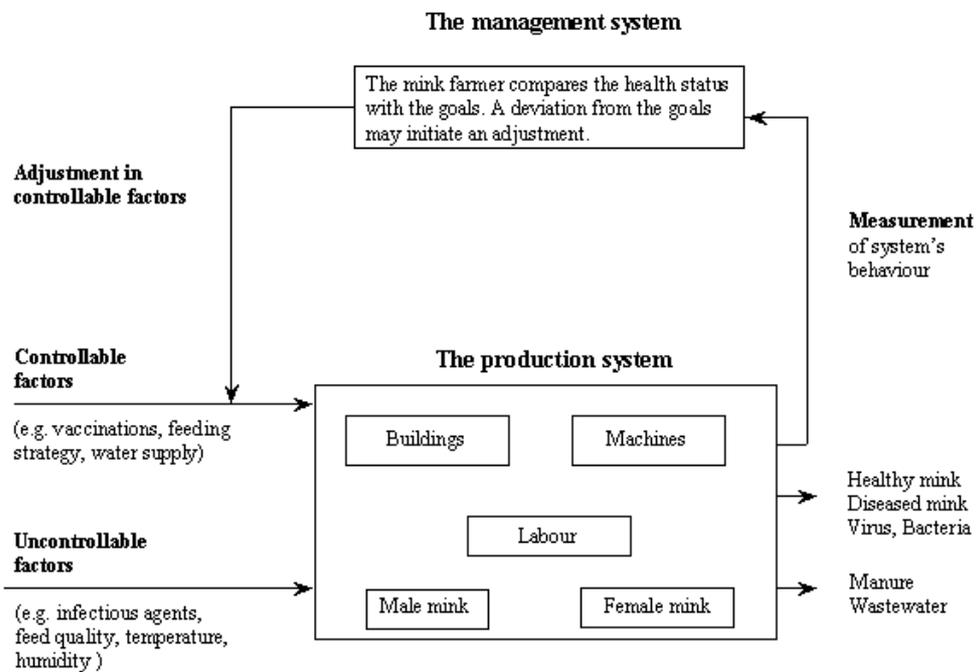
#### *Management*

Health management in a mink farm can be described as a cybernetic system (Sørensen & Kristensen, 1992; Møller & Sørensen, 2004). In this context the farm is organised as a production system defined by the animals, buildings, machines, land and labour, and a management system defined by feedback of information performed by the farmer (Fig. 2). It is an open system, as it produces animal products and by-products by use of controllable and uncontrollable inputs (e.g. climate, feed quality and ingredients, infectious diseases). By regulating the controllable factors the farmer tries to maintain the production in harmony with the goal while

adjustments are needed, when uncontrollable factors induce deviations from the goal. The interaction between the production system and the management system is illustrated in Fig. 2. Management is seen as a chronological series of: 1. Measurements of the production system's behaviour. 2. Comparison with a goal or a plan. 3. Adjustment in controllable factors

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

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



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

**Preventive health management and the application of HACCP**

*Vaccination*

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

*HACCP*

The principles of Hazard Analysis and Critical Control Point (HACCP) are developed for assuring food safety from harvest to consumption: "Preventing problems from occurring is the paramount goal underlying any HACCP system. Seven basic principles are employed in the development of HACCP plans that meet the stated goal. These principles include hazard analysis, CCP identification, establishing critical limits, monitoring procedures, corrective actions, verification procedures, and record-keeping and documentation.

Under such systems, if a deviation occurs indicating that control has been lost, the deviation is detected and appropriate steps are taken to re-establish control in a timely manner to assure that potentially hazardous products do not reach the consumer" (NACMCF, 1998).

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

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

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

#### *A hazard for pre-weaning diarrhoea*

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

#### Hazard analysis

##### Hazard identification

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

##### Hazard evaluation

Although the hazard and its occurrence in practice was identified, the severity and seriousness of the consequences could not be properly evaluated. E.g. the results by Møller & Chriél (2000) were based on very detailed registrations on 6 farms which was too

few for providing conclusive evidence regarding risk factors and odds ratios relative to other relevant factors. Therefore an epidemiological analysis at farm level has been performed based on production data from 5 years (1994-1998) from a total of 125 farms receiving feed from one of the largest mink feed producers in Denmark. Reproduction and herd size (number of female breeders on the farm) data were collected from the breeding system DanMink. Data on feed delivered to the farm as well as the number of mink on the farm was collected from the feed plant and the average feed allowance was calculated on a weekly basis for weeks 1 to 18 (from the beginning of January to the beginning of May). The occurrence and incidence of the pre-weaning diarrhoea syndrome was collected by an annual questionnaire. Four characteristic feeding periods were defined (weeks refer to the time period of the year, e.g. week 1 is the first week in January).

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

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

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

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

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

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

The risk factors were tested in a univariable logistic regression model with Farm as repeated effect between years. An autoregressive correlation structure was chosen because management of subsequent years seems more likely to be related.

Complete data from 125 breeders with 1-5 years of observations were analyzed. From 47 farms only one year with complete data was available, while only 9 farms had complete data from all 5 years.

The analysis showed that large difference in feed allowance between the Flushing and Conditioning periods and between the Implantation and Prenatal periods as well as herd size significantly increases the risk of pre-weaning diarrhoea at farm level. A difference between the Flushing and Conditioning periods of 90 kcal/female/day at farm level increases the risk (OR = 3.03;  $p=0.01$ ). By each kcal/female/day in difference between the Implantation and Prenatal two periods the risk of pre-weaning diarrhoea at farm level increases by OR=1,013 ( $p<0,05$ ). A difference between the two periods of 50 kcal/female/day at farm level increases the risk by OR = 1,91.

In other words, two hazards for pre-weaning diarrhoea at farm level have been identified: 1). Severely restricted feeding during the winter making room for a high energy allowance during the flushing and mating period. This result is in accordance with previous results (Olesen & Clausen, 1990; Møller, 1994; Møller & Chriél, 2000). 2). A high energy allowance during the implantation period and the first part of the gestation period followed by a drastic decrease in the later part of the gestation period. This confirms and quantifies previous results by Chriél (1994; 1997) and Møller & Chriél (2000; 2001).

#### CCP identification

“A critical control point is defined as a step at which control can be applied and is essential to prevent or eliminate a (food safety) hazard or reduce it to an acceptable level.” (NACMFC, 1998). Following the hazard evaluation the average feed allowance in kcal/female/day can be identified as Critical Control

Point for both hazards. The feed allowance may be calculated based on the amount of feed delivered from the feed kitchen and the number of mink on the farm, or by the energy content of the feed and the feed allowance fed to the mink. Another potential Critical Control Point could be the average weight or the weight change during the period

#### Establishing critical limits

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

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

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

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

### Discussion

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

allowance has been found to decrease the females' lactation capacity in the nursing period (Møller, 1994; Brzozowski & Møller, 1996) and to reduce the amount of mammary gland tissue 6 weeks post partum (Møller & Sørensen, 1999).

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

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

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II – 2 P

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

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### Abstract

The mink industry would benefit tremendously from linkage and cytogenetic maps of the mink genome, offering an opportunity to accelerate the rate of genetic improvement. The objective of this work was to create the first generation linkage map of the mink with at least 20 cM resolution, which will serve as a basis for further refinement. Genotypes of a mapping population consisting of four males, nine females and 71 F<sub>1</sub> progeny were determined at 46 microsatellite loci, of which 34 were informative and could be scored accurately. Six markers were assigned to two linkage groups using the Crimap software. Physical mapping of the microsatellites was also performed using a panel of mink-hamster hybrid somatic cell lines, showing consistent results with the linkage map.

### Introduction

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

using available microsatellite markers. Here we present the preliminary results of this first attempt to create a linkage map for the mink.

### Materials and methods

*The mapping population:* Seventy-one F<sub>1</sub> progeny from nine litters sired by four males constituted the mapping population. Two of the sire families were crosses between sapphire males and pearl females, and the other two were crosses between the Scand black and wild-type mink (mahogany). The main criterion for selecting a sire family was having at least 18 kits in three litters. Tissue samples (spleen and the lungs) were collected from sires, dams and kits after pelting.

*Laboratory procedures:* Genomic DNA was extracted from the spleens using a standard salting out method. Primer sequences for the amplification of 46 mink microsatellite markers by the polymerase chain reaction (PCR) were obtained from published sources (O'Connell *et al.*, 1996; Brusgaard, 1998; Fleming *et al.*, 1999; Vardy, 2003; Vincent *et al.*, 2003, 2004). Several of these microsatellites were originally developed for other members of *Mustelidae* family and were shown to amplify mink DNA (Davis and Strobeck, 1998; Fleming *et al.*, 1999). Published microsatellite primers for otter (Dallas and Piertney, 1998) and badger (Carpenter *et al.*, 2003) were used, and those that amplified mink DNA and were polymorphic in our mink families were used in this study.

The forward primers were fluorescently labeled with NED (Applied Biosystems), 6-FAM or HEX (TAG, Copenhagen, Denmark). PCR amplification was performed in 10 µL total volumes containing 50 ng genomic DNA, 1 µM of each primer, 1-2.5 mM MgCl<sub>2</sub>, 0.2 mM each dNTP and 0.25 unit of *Taq* polymerase (Roche, Laval, QC). All loci were

amplified using the touch down PCR protocol (start 64°C, 1° each step) followed by 33 cycles of 95°C for 15 sec, annealing temperature (55-60°C) for 60 sec and 70°C for 15 sec in an Eppendorf Master Cycler (Hamburg, Germany).

Polymorphism of each locus was determined by genotyping the parents and a few offspring. The entire mapping population was genotyped using all the informative markers. Genotyping was performed using an ABI 3100 DNA sequencer equipped with the GeneScan and Genotyper software (Applied Biosystems, Inc., Foster City, CA). Diluted amplicons and a size marker (500 HD ROX, Applied Biosystems) were denatured at 90°C for 3 minutes prior to loading (11 µL) onto 96 well plates. The Crimap software (Green, 1990) was used to perform two-point linkage analysis of the polymorphic loci.

### Results

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

### Discussion

The LOD scores indicated that the mapping population is useful in detecting linkages between

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

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

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

\* *Recombination frequency*

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

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

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II – 3 P

## Not diagnosed stage of Aleutian Disease

*V.S. Slugin*

### Abstract

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

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

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

### Introduction

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

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

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

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

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

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

\*-mink with signs AD.

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

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

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

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

### Material and Methods

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

### Results

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

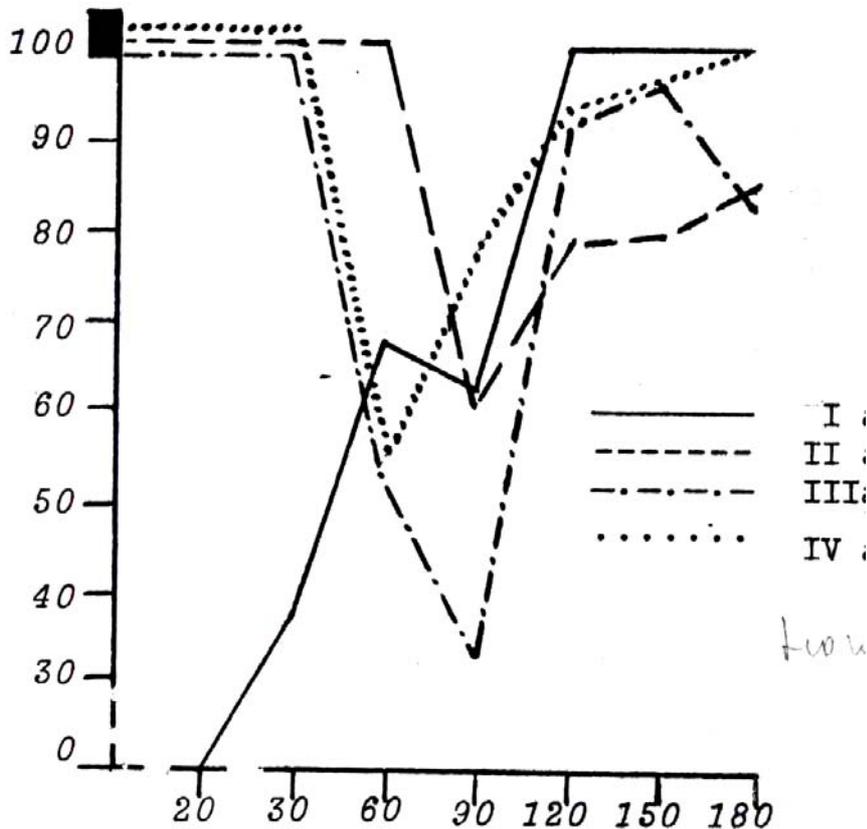
the kits from under infected of the mothers in the certain season petered positive CIEP (fig. 1).

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

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

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

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



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

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

Abaissements positive CIEP on 60- and the 90-th days of life of the kits, as it is visible from a fig. 1, are connected appreciably to a transplacental infection or with consumption of milk seropositive female, i.e. with colostric specific antibodies. The combination of consumption colostric antibodies with intraplacental exposure enlarges percent and duration of abaissements. The titration antibodies at

mink of these groups specifies also fall of a level antibody after weaning season. Thus the fall of titers antibody has not touched the kith I group.

### Discussion

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

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

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

from which it is planned to leave on breeding aims even one animal.

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

### Conclusions

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

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II – 5 P

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

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### Abstract

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

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

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

### Introduction

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

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

The Studying of marmots' reproductive activity is important not only in the theoretical plan, but also due to the connection with economic use of these

animals because the exposure of biological laws of dynamics of reproductive activity, spermatogenesis and estral cycles can increase the results of reproductive ability.

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

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

### Material and Methods

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

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

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

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

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

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

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

### Results and discussion

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

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

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

cytoplasm, a lot of mitochondrias and lipid inclusions of cytoplasm.

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

### Conclusion

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

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

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

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**Table 1. Weight of organs of marmots, g**

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

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

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

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

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

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

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

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

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

II – 6 P

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

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### **Summary**

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

### **Introduction**

Excess of aerobic radicals is hazardous for animal health owing to their high nonspecific reactivity. This may lead to the imbalance between antioxidants and prooxidants in favour of oxidation, that in a consequence induces the status called the oxidation stress. Numerous clinical examinations confirm a relation between the oxidation stress and health state disturbances [Kleczkowski et al.,1998; Saba et al.,1993; Saba et al.,1996]. The mentioned above state need not to be caused by a direct influence of free radicals, still it is of great importance in the whole chain of events giving rise to a polyetiological disease and development of the concurrent symptoms. Thus, the sources of free radicals seem to be vital as they are developed in organism under the endo- and exogenous

conditions. The latter include, among others nutrition, ultraviolet radiation and substances polluting the environment predominantly [Bartosz,2003; Benzie &Strain.,1996; Sitarska et al.,1997].

The objective of the present paper was to determine the values of some blood plasma markers considered the oxidation state indices at minks aged 1 year maintained at the definite farm conditions.

### **Material and Methods**

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

### **Results**

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

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

A vital role in the defensive system against the FR attack was performed by the antioxidative enzymes. They include, among others superoxide dismutase (EC-SOD) and glutathione peroxidase (GPx). The above mentioned enzymes metabolite free radicals ( $O_2$  in the case of SOD) or the half products ( $H_2O_2$ ) in the case of (GPx) into less toxic or nontoxic products. Activity of superoxide dismutase in the extracellular fluids is lower than in intracellular. Despite this, the cell surface is protected against superoxide anion radical by means of small quantity of EC-SOD bounded with them [Bartosz,2003]. Throughout the investigations, the EC-SOD level reached 20.05 – 10.23 U/l.

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

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

### Discussion

Glutathione participates in the reconstitution of the damaged cell components. It is noteworthy that its main function is to keep protein thiole groups reduced, which in many cases is simply essential for the functional activity of proteins. What seems interesting is a fact that its concentration drop to only half of the values regarded the references, as a rule does not lead to any noticeable physiological effects. Whatsmore, the enzymes interacting with GSH in a cell do not change their activity even under the conditions of a significant decrease of this

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

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

\*  $p \leq 0,05$ , \*\*  $p \leq 0,01$

tripeptide. It is only a considerable fall of GSH concentration that reduces their efficiency [Bartosz,2003]. In the carnivorous furry animals breeding, in that minks, there appears a special risk of animal organism exposure to the oxidation stress resulting from air pollution. It follows from a fact that furry animal farms emit some tens of odourforming substances, mainly sulphoorganic compounds, ketones, aldehydes, alcohols as well as aliphatic and aromatic hydrocarbons [Nowakowicz-Dębek et al., 2001; Saba et al.,1993; Saba et al.,1996].

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

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

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

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## Mink Nursing Sickness Survey in North America

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### Abstract

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

### Introduction

Nursing sickness is a metabolic disorder of large economic importance to the mink industry and represents the largest single cause of mortality in the adult female mink (*Mustela vison*). It is believed to develop from a complex of metabolic, nutritional and environmental factors, which influence the ability of the mink dam to meet the extreme demands of lactation (Clausen *et al.* 1992). Increasing dam age, large litter size, and female weight loss have been identified as major determinants for the development

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

### Materials and Methods

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

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

### Results

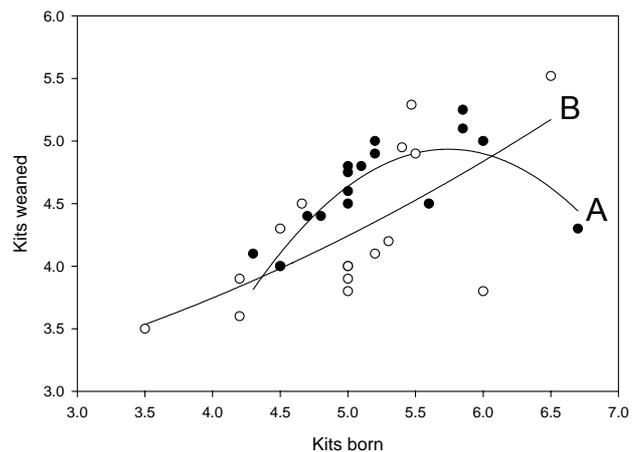
Overall, 52 ranches responded, consisting of 27 from Canada and 25 from the US. Of the total respondents, 13 surveys were found to be ineligible due to census errors (8 cases), non-response (4 cases) and changes in farm operation status (1 case). Census error refers to those surveys not clearly indicating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

#### Ranch History

The survey respondents housed a total of 99,333 breeders females of which 56% represented the black, 31% the brown, 8% the blue and 5% other color types of mink. The pelt production of these farms totaled 393,717 pelts. Forty-five (45) % of the ranches surveyed reported problems with nursing sickness, of which 87% encountered them every year. Thirteen (13) % of these farms had 10-40% of their females affected, 17% had an incidence rate of 5-9%, and 70% had less than 4% of the females with nursing sickness. Conclusions could not be made regarding color type and occurrence of the disease, as census responses did

not differentiate these relationships. The onset of the disease was seen typically around 30-39 days (44%) or after 40 days (28%). Schneider and Hunter (1992) report typical onset of the disease at around 42 days after whelping. The farms reporting problems with nursing sickness had a higher average litter size per female on ranch at birth (5.2) in comparison to farms without nursing sickness (5.0). A trend toward higher average number of kits weaned was found on affected ranches (4.6) in comparison to those without nursing sickness (4.3,  $P=0.096$ ) (Figure 1).

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



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

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

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

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

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

causing the female potentially to experience higher levels of (heat) stress.

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

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

#### *Animal Management*

The main animal management practices surveyed among the healthy and affected ranches are presented in Table 2. On healthy farms, the main breeder female selection criteria used in November focused more on body length (healthy 83%, nursing-sickness 62%,  $P=0.091$ ), whereas body weight was more emphasized on the farms with problems (healthy farms 17%, nursing-sickness farms 43%,  $P=0.062$ ). Selection for body weight was also found to be more prevalent in February on ranches experiencing problems with the disorder (healthy 0%, nursing-sickness 29%,  $P=0.052$ ). One hundred percent of ranches affected by nursing-sickness reported conditioning of breeder females, compared to 78% of healthy farms ( $P=0.037$ ). Of the farms that practiced conditioning, 95% of those experiencing nursing sickness began conditioning between January and February, compared to 15% of healthy farms beginning between September and December, and 77% between January and February ( $P=0.065$ ). Methods of conditioning also varied between the two

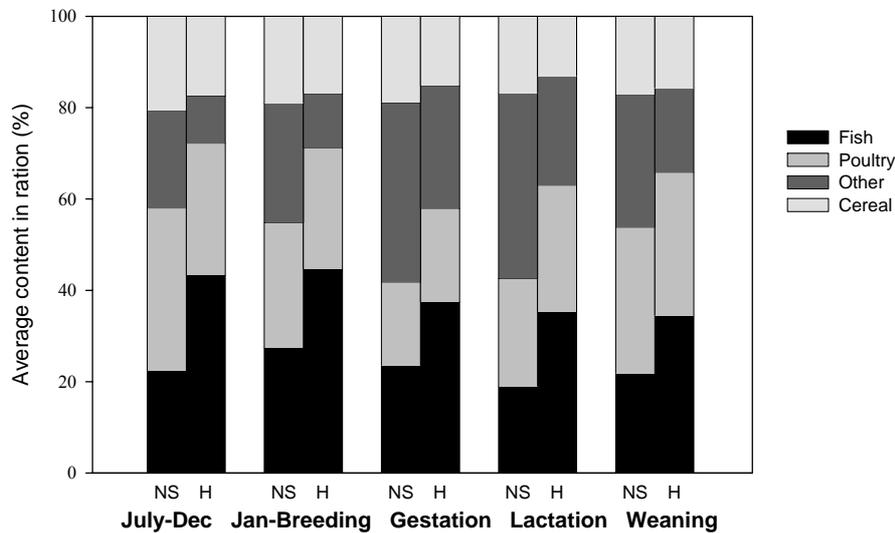
groups ( $P=0.049$ ). Eight (8) % of healthy farms tried to keep their females active, whereas none (0 %) of the nursing-sickness ranches employed this practice. On the healthy farms, reduced feed allowance was practiced by 69% and dietary fat content was reduced by 15% of the respondents, while 59% of the nursing-sickness farms reduced the amount of feed and 29% reduced the amount of fat. Both groups marked thin or obese females (healthy 8%, nursing-sickness 12%). It is apparent, that selection for body length is likely associated with a more lean body type, whereas selection for body weight may result in heavier body condition and thus more body fat. In carnivore companion animals, both body fat excess (obesity) and low physical activity are associated with poor glycemic regulation (Burkholder & Toll, 2000, Zicker et al., 2000). The farms selecting their breeder females based on body weight and not encouraging exercise may inadvertently impair the females' ability to regulate blood sugar levels, causing disruption in glucose homeostasis (Børsting & Gade, 2000).

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

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

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

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



#### *Feed Composition and Feeding Management*

The feed composition differed greatly between the farms. The farms without nursing sickness problems fed much more fish in their diet (34-42.5%) throughout the production year than the farms, which encountered problems (18-27%) (Figure 2). Through July to December (growing and furring) average fish content was found to be significantly higher in the apparently healthy farms (42.5%) than in the affected farms (22%)( $P=0.049$ ). Differences were also observed in the dietary content of other animal by-products (i.e. not fish or poultry) during this period (healthy 10%, nursing-sickness 21%,  $P=0.038$ ). The amount of other animal by-products fed differed also at breeding (healthy 20%, nursing-sickness 35%,  $P=0.004$ ), gestation (healthy 26%, nursing-sickness 38%,  $P=0.061$ ), and lactation (healthy 23%, nursing-sickness 39%,  $P=0.040$ ). The average content of cereal, fish, poultry and other animal by-products in the diet during the remaining phases of production were not found to differ between healthy and affected ranches. Dietary n-3 fatty acids, commonly found in fish, have been shown to improve glucose transport and metabolism resulting in improved glucose tolerance (Takahashi and Ide 2000). It is likely that the higher amount fish, providing more of the n-3 fatty acids, fed on the ranches not experiencing problems with nursing sickness may help the mink

females to better regulate their blood sugar levels during lactation thus preventing the occurrence of the metabolic disorder of nursing sickness. Although the largest difference in the level of fish in the diet was observed during the fall months, the dietary background of the female will have long-lasting impacts influencing the composition of both body and milk fat during the nursing period. These results support the suggestion that a deficiency may develop in the lactating mink particularly during the latter part of the nursing period due to the substantial secretion of the n-3 fatty acids in the milk (Rouvinen-Watt 2003). The high physiological demand for the n-3 fatty acids during lactation may result in poorer glucose tolerance in females, which do not have an adequate dietary supply or have not accumulated adequate quantities of the n-3 fatty acids in their body fat reserves.

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

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

### Conclusions

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

### Acknowledgements

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II – 8 RP

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

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### Abstract

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

### Introduction

In the newly proposed hypothesis for the etiology of nursing sickness in the mink (*Mustela vison*) (Rouvinen-Watt, 2003), striking similarity is suggested between the clinical symptoms seen in the affected mink dams and those observed in the metabolic syndrome associated with acquired insulin resistance. Type 2 diabetes, or non-insulin dependent diabetes mellitus (NIDDM) is a disorder characterized by insulin resistance or abnormal insulin secretion

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

### Materials and Methods

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

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

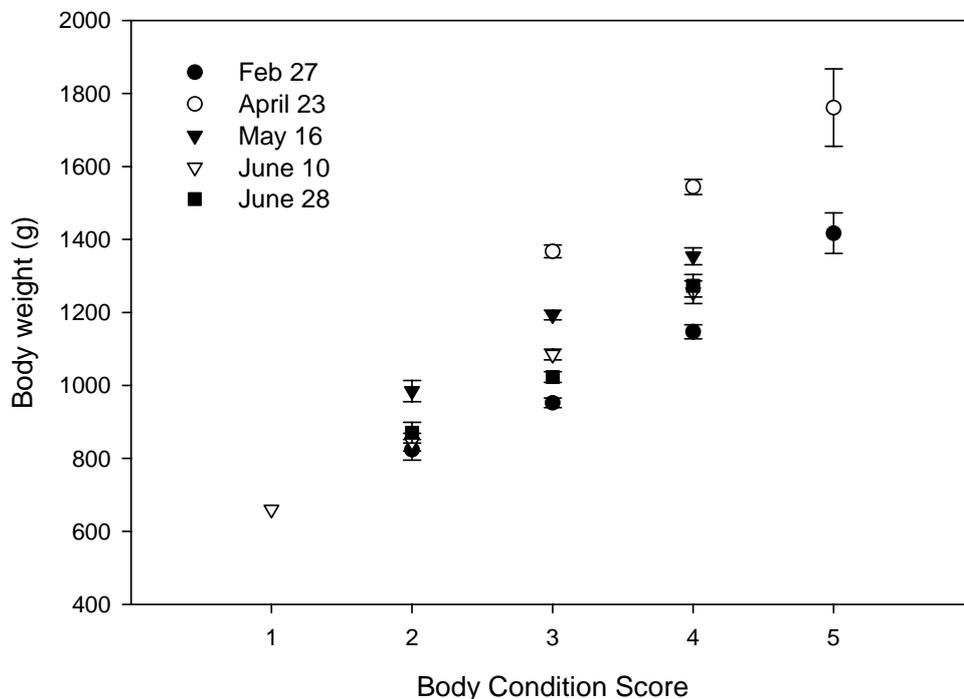
**Materials and Methods**

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

**Results and Discussion**

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

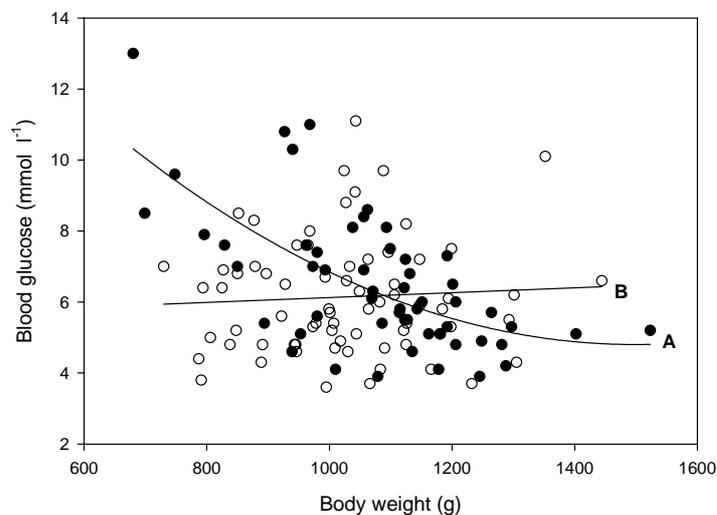
**Figure 1. Relationship between body condition score and body weight of mink breeder females from breeding (late February) until one week after weaning (June 28). The regression equation for February is  $y = 393.4 + 197.6x$  ( $R^2=0.98$ ), April:  $y = 769.7 + 196.9x$  ( $R^2=0.99$ ), May:  $y = 622.7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



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

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

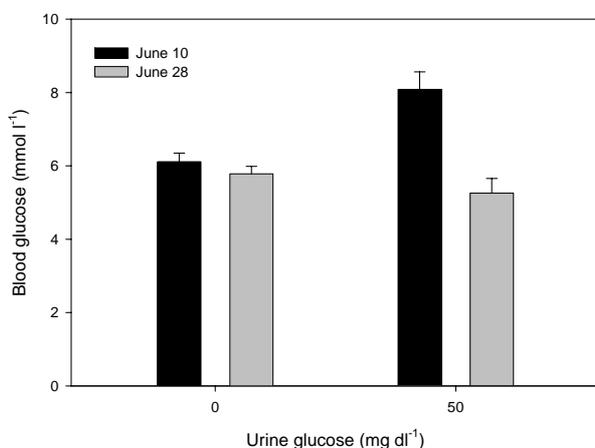
**Figure 2. Relationship between body weight and blood glucose concentration of mink breeder females at late lactation (June 10) (●) and after weaning (June 28) (○). The regression equation for curve A (before weaning) is  $y = 23.30 - 0.02x + 0.00000824x^2$  ( $R^2=0.40$ ), and for line B (after weaning)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



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

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

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



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

### Materials and Methods

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

### Results and Discussion

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

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

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

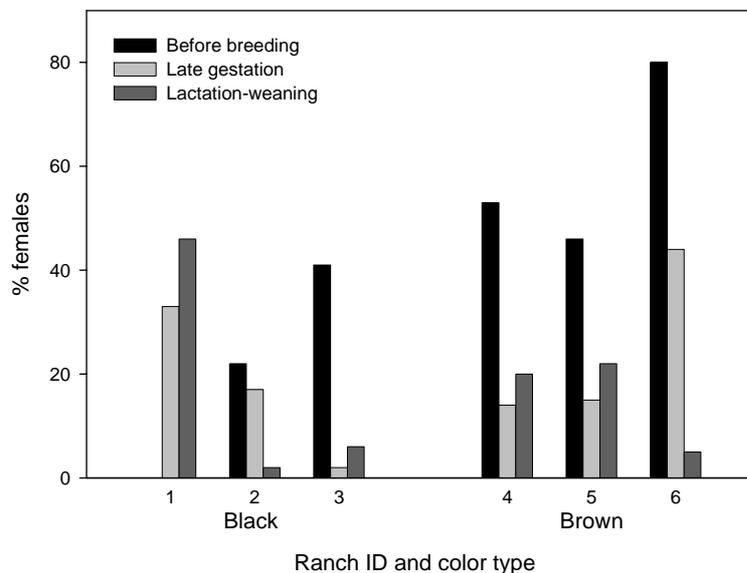
breeding, to close to 400 at subsequent sampling periods.

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

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

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

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



### Conclusion

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

### Acknowledgements

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Appendix A. Body Condition Scoring System

**Canadian Center for Fur Animal Research  
Nova Scotia Agricultural College**

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**BODY CONDITION SCORING OF MINK  
USING A FIVE-POINT SCALE**

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**SCORE 1. Very thin**

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

**SCORE 2. Thin**

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

**SCORE 3. Ideal**

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

**SCORE 4. Heavy**

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

**SCORE 5. Obese**

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

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

II – 10 RP

## Wet kits in mink, a review

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### Summary

“Wet kits” (also known as “greasy kits” or “sticky kits”) in mink is a multifactorial disease in the lactation period with few known definitive releasing factors. The disease is known in all mink-producing countries in the northern hemisphere, and has been observed on commercial mink farms in Denmark for more than 40 Years (Svennekjær, 1954).

The definition of “wet kits” is when mink kits develop a greasy, sticky exudate on the skin surface especially in the neck, and tail, as well as on the claws, a red and swollen perianal region, frequently a yellowish-white diarrhoea and invariably a mewing, distressed behaviour.

The effects of bacteria, virus, management, feed, immunology of the animals and environmental factors on “wet kits” are discussed.

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

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

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

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

### Introduction

“Wet kits” is a problem of great economical importance for mink breeders in Denmark. The number of farms affected annually varies considerably and can be very high. The morbidity rate can vary from 0 to more than 30 percent, and

the mortality is normally one to two kits per litter. Apart from the loss of kits a lot of time is spent on treatment and the medication can be rather expensive. Kits surviving the disease have a lower weight at weaning than unaffected kits, but the same weight and skin length at pelting (unpubl. obs).

A number of eliciting factors have been tested in Denmark in usually unpublished investigations. It has turned out to be very difficult to perform prospective as well as experimental studies of “wet kits” due to the high variation in annual morbidity rate. Furthermore, there is no obvious pattern in disease outbreaks among farms.

### Bacteria

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

No clear epidemiological evidence of an infection spreading on the farm during outbreaks of wet kits has been found so far (Chriél et al. 1997).

### Virus

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

(1987). Svansson (1991) found that intraperitoneal inoculation of a reovirus-like virus could cause symptoms resembling “wet kits”, and Järplid and Meyerland (1998) found histological alterations in the intestinal villus epithelium of wet kits resembling changes caused by rotavirus infection in neonatal calves.

Englund et al. (2002) found that astrovirus was a significant risk factor in the development of pre-weaning diarrhoea. Other factors, i.e. low body weight, coccoid bacteria adherent to enteric villi, and the presence of calicivirus, were also shown to increase the risk of pre-weaning diarrhoea.

### **Management**

In mink production management problems as a contributing factor in the development of “wet kits” has not been very well investigated so far.

A questionnaire revealed that there were bigger problems on large farms in Denmark (Olesen & Clausen 1990), probably due to a high population density. It has also been shown that the frequency of wet kits in groups with an empty cage between the females was significantly lower than in groups with a female in each cage (Overgård, 2000).

Many other factors have been discussed as contributing to the onset of the disease. Stressing the females by feeding on top of the nest box early in the lactation period, too much handling of the animals, hot weather that causes the females to leave the kits, inadequate sanitation and hygiene in the nest box etc. are potential factors.

Flushing is often applied to female mink prior to breeding (Atkinson, 1996). Some mink farmers, however, tend to reduce the energy intake too much and an epidemiological examination of large data sets have shown that a low energy intake in late April predisposes to “wet kits” (Chriél, 1997).

### **Feed**

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

The quality of the feed most probably will also be a contributing factor, but is not easy to prove.

### **Animals**

Hunter & Schneider (1996) postulated that “wet kits” or adenitis of the neonatal cervical gland occurs frequently in mutation colour phases of mink. Our experience is that all colour types are affected but the most serious outbreaks are usually seen in black mink and blue mink.

Litters with many kits from young females giving birth late in the period are at greatest risk (Olesen & Clausen, 1990). So far, inheritance has not been shown as an important factor.

A few days after birth, the kits have the same amounts of antibodies against virus enteritis (parvovirus) in the blood as the female, independent of the number of kits in the litter (Uttenthal et al., 1999). The amount of antibodies in serum and colostrum in heifers is lower than in cows (Radostits et al. (1994), and if this is the same for young female mink, it may contribute towards making their kits more susceptible to the disease.

Kits born late will be disposed to a greater amount of infectious agents than kits born early in the period, thereby increasing the risk of “wet kits” for late born litters.

### **Mastitis**

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

### **Milk**

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

wet kits and females with healthy kits has been shown.

### Conclusion

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

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

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

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

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## Oral immunization of fur-bearing animals against salmonellosis

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### Abstract

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

### Introduction

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

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

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

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

### Materials and Methods

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

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

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

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

phagocytic reaction field virulent strain *Sal. typhimurium* was used as a test object.

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

### Results and Discussion

A vaccine-feed mixture was eaten by animals fully and willingly. And changes in animals' behaviour, drop of appetite, refusal of feed, vomiting, alimentary canal upset, signs of depression and disease of animals were not noted. Even when vaccine doses were many times higher than an

immunizing one, animals remained clinically healthy and active. Immunization of females did not have a negative impact on the course of pregnancy and normal development of the offspring.

The results of immune reaction studies are given in Tables 1 and 2. To compare the results those tables contain data on parenteral administration of vaccine from attenuated strains.

When studying postvaccine changes in the organisms of animals it was found that different methods of vaccination resulted in the same dynamics of the immune response. Immune indices increased already on the 7 th day after vaccination, their maximal values were noted on the 14 th day. Then their gradual decrease took place. Practically during all the dates there was a certain increase both of specific antibody titers in blood serum of vaccinated animals, and the indices typical of phagocytic activity of leukocytes. Besides, it is

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

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

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

Groups of Test Animals (n=5)	7 days		14 days		21 days		28 days		
	M± m	P	M± m	P	M± m	P	M± m	P	
<b>Fox</b>	Parenteral	32.6± 2.27	< 0.001	36.2± 2.33	< 0.001	32.6± 0.93	< 0.001	28.5± 0.86	< 0.001
	Oral, double	32.0± 3.18	< 0.02	34.0± 2.87	< 0.001	30.2± 52.0	< 0.01	26.2± 1.65	< 0.02
	Control	19.4± 1.36		18.5± 1.5		19.4± 1.36		17.7± 1.8	
<b>Arctic fox</b>	Parenteral	36.0± 6.46	< 0.02	34.2± 2.85	< 0.001	29.5± 2.5	< 0.01	27.5± 1.5	< 0.001
	Oral, double	30.0± 1.22	< 0.001	29.2± 1.89	< 0.001	27.7± 0.95	< 0.001	23.5± 2.7	> 0.5
	Control	16.5± 1.36		17.5± 0.33		19.5± 1.44		21.0± 0.02	

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

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

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

It was shown that on the basis of investigations carried out earlier (Domski, 2003) titers of specific antibodies in all dates of studying indicated a rather strong immunity to salmonella infection, and it was proved that their level saved the Arctic fox young from disease and death when control infecting with virulent strains of pathogenic organisms took place. Indices of an opsono-phagocytic reaction showed a strongly pronounced cell immune reaction to the virulent strain and that reaction characterized specificity and trend of immune changes in the organism of fur-bearing animals vaccinated against salmonellosis.

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

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

### Conclusion

The results of studies showed that there were no contraindications for oral immunization of animals

against salmonellosis. It was proved the innocence and areactogenicity of a new vaccine.

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

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

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