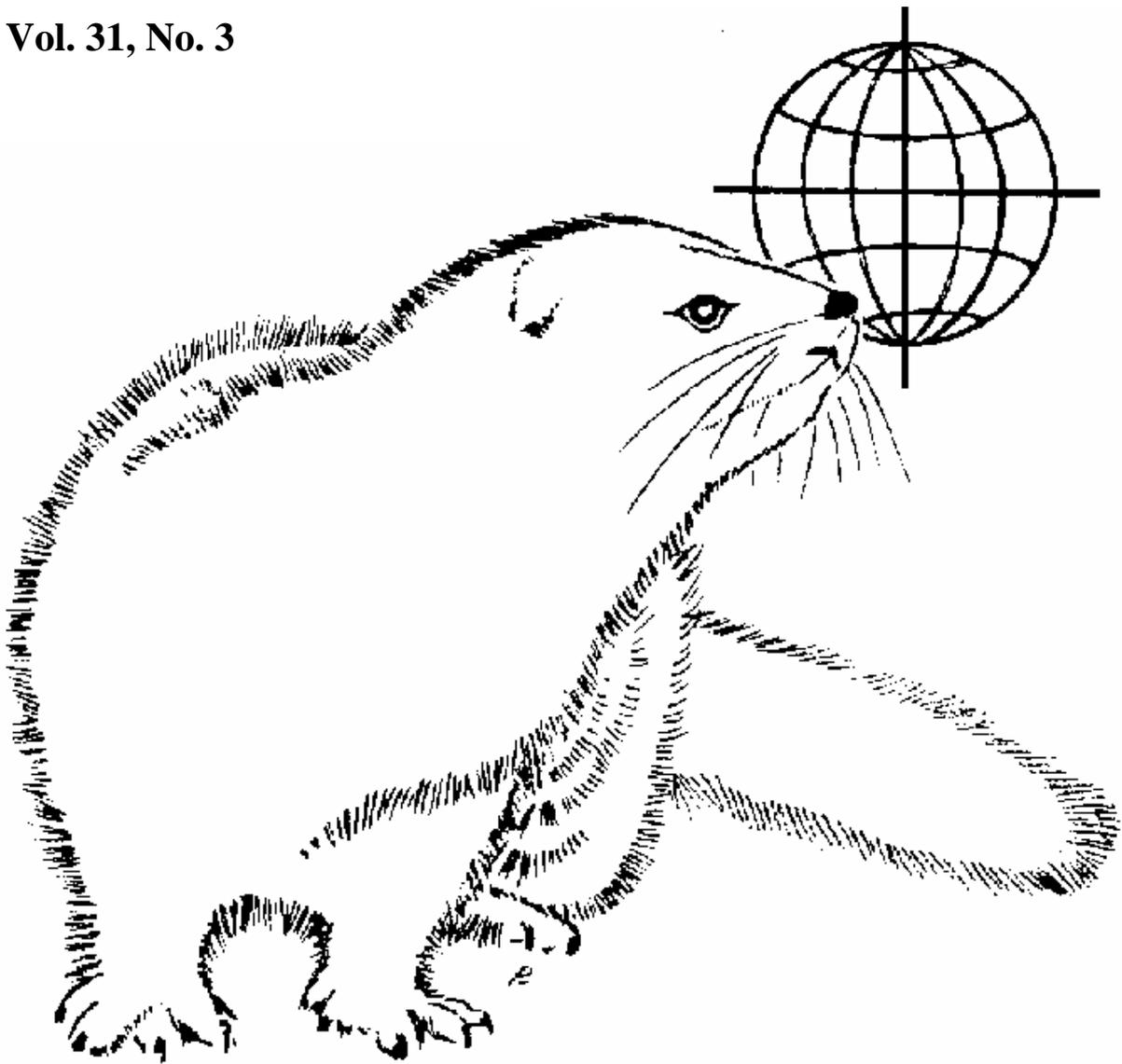


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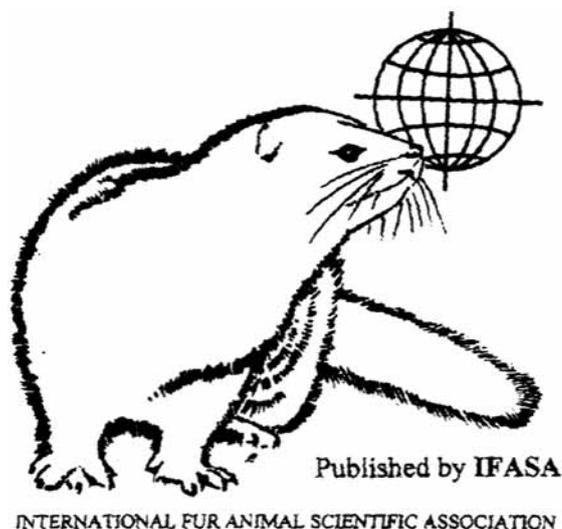
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Notes from the Group of Editors

This issue of Scientifur, Volume 31, No 3, contains a number of abstracts provided by one of our local representatives, an interesting reviewed article, as well as the abstracts of the well attended NJF Seminar No 403, which was held in Kolding, Denmark, 13-15 August 2007.

We are grateful to our Scientifur representatives for their provision of information and are pleased that the Board of Directors of the IFASA has recently

appointed more representatives (their names are listed on the inside of the front page).

The fact that we have changed the publishing procedure in relation to reviewed articles (they are no longer published once a year in a special issue of Scientifur) has reduced our publishing times markedly, and we hope you will find that this has been to the benefit of our authors as well as our readers.

On behalf of the
Group of Editors

Birthe Damgaard

The effects of estradiol, progesterone and prolactin on mink uterine luminal and glandular epithelial cell growth

D. Rasmussen, D. Mecham, J. Thomas, J. Rose

The hormones 17beta-estradiol (E2), progesterone (P4) and prolactin (PRL) have been shown to have direct actions on the uterus of many species that are essential for preparing the organ for embryo implantation. Our objectives were to determine the effects of E2, P4 and PRL on uterine luminal and glandular epithelial cell growth in anestrous mink, an animal for which such knowledge is lacking. Mink were treated with E2, P4 or haloperidol (HAL; a dopaminergic antagonist that increases PRL secretion), alone or in combination. Histological images of the uterus in cross section were digitized and cell heights measured using Image J software (NIH). E2 or P4 increased luminal and glandular cell height relative to controls ($P < 0.001$). Glandular cell height was approximately doubled in response to P4 when compared to control or E2 treated mink ($P < 0.001$), and E2+P4 increased glandular cell heights, compared to E2 alone ($P < 0.001$). Interestingly, HAL caused luminal and glandular cells to be taller than controls ($P < 0.05$). When combined, HAL+E2 increased glandular cell height to a greater extent than in response to E2 alone. Glandular cell height was taller in response to HAL+P4 than HAL+E2 ($P < 0.001$). Luminal cell height in response to HAL+E2+P4 was greater than HAL, HAL+E2 or HAL+P4. Because blood PRL levels increase throughout the breeding season of mink, we hypothesize that PRL, in combination with E2 and P4, plays an essential role in creating an optimal uterine environment for blastocyst implantation.

49th Annual Meeting and Symposium of the Idaho Academy of Science, 19-21 April 2007.

Effects of estradiol, progesterone and prolactin on glycogen accumulation in the mink uterus

E.D. Mecham, J. Thomas, J. Rose

Most mammalian prenatal losses occur after fertilization, but before implantation, emphasizing that optimal uterine conditions must be established to ensure reproductive success. The uteri of rats,

mice and humans accumulate glycogen as an energy source, in a reproductive cycle-dependant pattern. And, many reproductive failures (ie. spontaneous abortions), are correlated with low uterine glycogen concentrations. Our objectives were to determine: 1. if the mink uterus contains glycogen as an energy source, and 2. the effects of estrogen, progesterone and prolactin (PRL) on uterine glycogen accumulation. Female mink (4 /group) were assigned at random to one of 8 groups. Mink in Group 1 received no treatment (Controls). Mink in Groups 2, 4, 6 and 8 each received a slow release Silastic implant containing 100 mg estradiol-17beta (E), while mink in groups 5, 6, 7, and 8 each received an implant containing 200 ug Haloperidol (HAL) to increase endogenous PRL secretion. One week later, mink in groups 3,4,7 and 8 each received an implant containing 100 mg progesterone (P). Mink were sacrificed one week later and uterine samples assayed for glycogen concentrations by glucose hexokinase chromophotography assay. Both E and P increased glycogen concentrations ($P < 0.05$), but exhibited no synergism when given together. Interestingly, HAL-treated mink, exhibited significantly elevated uterine glycogen concentrations ($P < 0.05$), and mink treated with HAL + E had the greatest concentrations of glycogen, suggesting a synergism between E and PRL. It would now appear that in addition to their many other direct actions on the uterus that are essential for implantation, uterine glycogen synthesis is also stimulated by these hormones in mink. Because blood PRL levels increase in mink prior to and during the breeding season, we hypothesize that PRL and E may promote the development of a more receptive uterine endometrium for implantation, in part, by increasing uterine glycogen reserves.

Idaho Chapter of the Wildlife Society, 5-7 March 2007.

Effects of estradiol, progesterone and prolactin on growth of the uterine myometrium of mink

H. Amen, D. Mecham, J. Thomas, J. Rose

Our objectives were to determine the effects of exogenous 17β-estradiol (E2), progesterone (P4) and prolactin (PRL) on growth of the uterine

myometrium of anestrous mink. Adult female mink (3 /group) of proven parity, were treated with slow release implants containing E2, P4 or haloperidol (HAL; a dopaminergic antagonist that increases PRL secretion), either alone or in combination. At the end of three weeks, all animals were sacrificed and 10 μm cross-sections of one uterine tube prepared from each animal and stained with Hematoxylin and Eosin. Myometrial area (μm^2) was determined for each animal (N=3 measurements per animal) using Image-J software (NIH) and expressed as a ratio of the total uterine area. Exogenous E2 significantly increased myometrial area relative to controls. Treatment with P4 had no effect on myometrial growth and showed no synergism with E2. Interestingly, exogenous HAL significantly increased myometrial area when compared to controls ($P < 0.01$). HAL showed no synergism with E2 or P4 either alone or in combination. The major finding of this study is that PRL appears to have an independent action on the mink uterus, stimulating growth of the myometrium. Because blood PRL levels increase throughout breeding, fertilization, implantation and parturition in mink, we hypothesize that PRL may contribute to successful parturition by promoting myometrial smooth muscle growth during pregnancy. This action could contribute to a greater number of successful live births and thus increased litter sizes.

49th Annual Meeting and Symposium of the Idaho Academy of Science, 19-21 April 2007.

The effects of catecholestrogens on glycogen production and cellular distribution in the rat uterus.

J. Thomas, D. Mecham, J. Rose

Glycogen, produced by the uterus, is a potential source of energy for fertilization and embryo implantation. Progesterone and 17 β -estradiol (E2) stimulate uterine glycogen production in rodents and humans in a reproductive cycle dependent manner. In humans, uterine glandular glycogen content increases near the time of implantation. Indeed, infertility and spontaneous abortions are associated with low uterine glycogen content. The uterus metabolizes E2 to catecholestrogen's (CE's) such as 2-

hydroxyestradiol (2-OHE2) and 4-hydroxyestradiol (4-OHE2). In rodents, 4-OHE2 acts directly on the blastocyst to promote implantation. However, it is not known if CE's influence uterine glycogen metabolism. Our objectives were to determine the effects of 4-OHE2 and 2-OHE2 on uterine glycogen concentrations and cellular distribution in parous (PAR) and nulliparous (NULL) rats. Twenty-four PAR and twenty-four NULL rats were assigned at random to one of four groups (N = 6/group): 1 = Controls, 2 = E2, 3 = 4-OH E2, and 4 = 2-OH E2. All rats were bilaterally ovariectomized and beginning ten days later were injected with either E2, 4-OHE2, or 2-OHE2 (250 micrograms/kg BW) in sesame seed oil twice daily for three days, while Control rats received oil injections only. Twelve hours after the last injection, rats were sacrificed and uteri collected. Total uterine glycogen concentrations were determined using Infinity® Glucose Hexokinase liquid Stable reagent to quantify glucose concentrations before and after glycogen hydrolysis. Cellular localization of glycogen deposits were determined using periodic acid Schiff staining. In PAR rats, uterine glycogen concentrations (mg/g dry weight, + S.E.M.) were greater in response to E2 (22.59 + 2.38) and 4-OHE2 (19.72 + 2.38) relative to controls (2.02 + 1.67; $P < 0.001$), and although glycogen concentrations tended to be higher in response to 2-OHE2 (7.29 + 1.83), differences were not significant. In NULL rats, glycogen concentrations were not significantly different between any of the groups, although the trends were similar to PAR rats (Control = 4.08 + 0.69, E2 = 7.42 + 1.45, 4-OH E2 = 7.92 + 1.87, 2-OH E2 = 4.24 + 1.49). Glycogen staining was greater in the stroma than in luminal or glandular epithelia of PAR and NULL Control rats. In both types of epithelia, staining intensity was greatest in the apical regions of the cells. Exogenous E2 and 4-OHE2 increased glycogen staining in stroma as well as glandular and luminal epithelium of PAR and NULL rats. Interestingly, 4-OHE2 produced the greatest glycogen staining in the glandular epithelia of both PAR and NULL rats, when compared to luminal epithelia or stroma. The effects of 4-OHE2 on glandular cells was even greater than E2. Glycogen staining in response to 2-OHE2 was only slightly greater than controls. These findings illustrate that the uteri of PAR rats produce greater amounts of glycogen in response to E2 and 4-OHE2 than NULL rats. This apparent heightened sensitivity of the PAR rat uterus might, in part, explain why second pregnancy litter sizes are

greater than those of the first. Finally, 4-OHE2 may act in an autocrine/paracrine manner to influence the composition of uterine secretions that support blastocyst survival and implantation.

Annual Meeting of the Society for the Study of Reproduction. San Antonio, TX. 21-25 July 2007.

Anticipatory behaviour in mink reflects the rewarding properties of enrichments as well as the effect of enrichments on reward sensitivity

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Abstract

Sixteen farm mink were used in three consecutive experiments. All experiments involved training of the mink in a Pavlovian conditioning paradigm and testing them with a 10 min interval between the onset of the conditioned stimulus, a sound, and the presentation of the reward. The anticipatory behaviour in this interval was quantified as the total number of behavioural transitions. In the first experiment, all mink were subjected to the same housing conditions and the reward consisted of access to either a barren or an enriched environment. Access to the enriched environment elicited more behavioural transitions in the animals than access to the barren environment. This is suggested to be due to a higher rewarding value of this environment. In the second experiment half of the mink were housed in barren environments and the other half in enriched environments. The reward consisted of cat food served in a neutral part of the environments. The number of behavioural transitions was highest in the group of mink from the barren environments. This is suggested to be due to a higher reward-sensitivity caused by the less stimulating environment. The third experiment was conducted the same way as the second one; however, the two groups of mink were interchanged. Once again, the number of transitions was highest in mink from the barren environments. This shows that the effect of the environments on the anticipatory response is brought about in the inter-test interval of about 6 weeks. The results are discussed with respect to the relationship between reward-sensitivity and welfare.

It is suggested that the anticipatory behaviour reflects the balance between negative and positive experiences in the mink and by that the welfare state of the animals. The results thus show that mink living in enriched surroundings experience better welfare than the mink living in barren surroundings.

Keywords: Mink, Welfare, Enrichments, Anticipatory behaviour.

Introduction

Recently it has been argued that the welfare of an individual is determined by the balance between positive and negative experiences and affective states (Spruijt et al., 2001; van der Harst, 2003) and that animals develop behavioural strategies in order to maintain an optimal state of this balance. The mechanism of maintaining the balance is thought to be an experience dependent setting of the sensitivity of the reward system of the brain. Behavioural responses leading to experiences that are positive for the animal (e.g. in fulfilling biological needs) results in lower sensitivity to rewards while experiences that are negative for the animal (e.g. failing fulfilment of needs) increases the sensitivity. Fulfilling needs through adaptive behavioural actions with a minimum of effort requires a continuously changing sensitivity to rewarding and aversive stimuli. Spruijt et al. (2001) also argued that behavioural activation in anticipation of the arrival of a reward represents an activation of the reward system of the brain and that observing behaviour during anticipation of a reward in a

Pavlovian conditioning paradigm, therefore, may indicate the state or the sensitivity of the balancing system of the organism.

Van der Harst et al. (2003a, 2003b) confirmed that rats living in enriched surroundings, allowing a fulfilment of biological needs, reduces the sensitivity to rewards as reflected by reduced levels of anticipatory behaviour observed in a Pavlovian conditioning paradigm. On the above basis van der Harst (2003) argued that anticipatory behaviour could be used as a means of measuring welfare.

Van der Harst (loc. cit.) found that anticipation of a positive reward was most reliably reflected by increased frequency of transitions between distinct behavioural elements. Although focusing on the duration of specific anticipation (behavioural) elements, Hansen & Jeppesen (2006) showed that farm mink react in the same way as rats in anticipation of a positive reward, that is, by increasing their activity. In addition it was shown that anticipation of a negative reward reduced activity. On the above basis farm mink should have the potential for expressing their reward sensitivity or their state of welfare through anticipatory behaviour.

Vinke et al. (2004) used the anticipation measure to evaluate the effect of three different housing systems on the welfare of farm mink, and failed to show any effect of these housing systems, although they represented quite large differences in the amount of environmental enrichment. The result was tentatively explained by the fact that none of the housing systems were barren enough to increase the sensitivity of the reward system and thereby increase the anticipatory behaviour. Naturally, another explanation would be that the measure of anticipatory behaviour for the assessment of the impact of different housing systems is not discriminative enough in mink, and this possibility was examined in the present study.

The study consisted of three experiments with the overall aim of assessing the usefulness of anticipatory behaviour in farm mink as a tool to: 1) assess the rewarding value of two different rewards (anticipation of access to an enriched cage was expected to elicit more anticipatory behaviour than anticipation of access to a barren cage), 2) assess the welfare state of differently housed mink (mink housed in enriched cages were expected to show

less anticipatory behaviour in response to a standard feed reward than mink housed in barren cages), and 3) assess whether it is possible to change the reward-sensitivity of individual mink by changing their cage environments. In all three experiments the anticipatory response was induced by a Pavlovian conditioning schedule, and measured as the total number of behavioural transitions in the interval between the onset of the conditioned stimulus and the provisioning of the reward.

Materials and methods

All experiments in this study were performed at a private mink farm, Zealand, Denmark and took place from the beginning of December 2005 to the end of May 2006. The experimental mink were housed in roofed standard sheds with open sides. Each shed contained two parallel rows of 120 standard cages with nest boxes.

Animals and general housing

The experimental animals consisted of 16 wild-coloured farm mink females (*Mustela vison*) all of approximately same weight (1400-1600 g). They were pair wise sisters and were born in 2005 in the period April 18 – May 5. They had free access to drinking water and were fed once a day at 15:00 h with commercial mink food. Every morning leftovers were distributed among animals without leftovers. The mink were born in and raised in cages of standard size (45cm × 30cm × 90cm) with attached nest boxes (19cm × 30cm × 23cm). All training and testing of the animals was performed by the same person.

Experiment 1

In this experiment the anticipatory response of mink expecting access to an enriched cage was compared to the anticipatory response of the same animals expecting access to a barren cage.

- experimental housing

The 16 mink were housed in a central standard cage (including nest box) with two openings – one opening towards an enriched cage (left) and one towards a barren cage (right). The openings could be opened and closed by wooden partitions. The enriched cage consisted of a standard cage with a nest box supplied with extra straw, and the cage was supplied with 1-2 plastic balls, 1 floater for fishing nets, 1 sisal robe hanging from the ceiling, 1 net-cylinder, and/or 1 plastic cylinder. The diameter and

the length of the cylinders were 115 mm and 330 mm, respectively. They were both lying at the cage floor and sometimes they were filled with straw. The barren cage consisted of a standard cage without nest box.

Before beginning the conditioning procedure, all animals were allowed to move freely between the three cages for two days. This should make them comfortable with the different cage environments and make sure the animals could localise the enriched and the barren cage respectively. Subsequently the animals were placed in the central standard cage for two days without access to the other cages.

- conditioning procedure

A Pavlovian conditioning paradigm was used by which a reward (unconditioned stimulus, US: access to either enriched or barren cage) was announced by an initially neutral stimulus in the form of a 3 s humming sound. The training of the animals was conducted in the period December 5 – December 31, 2005. The daily training started with different animals to avoid systematizing. Every second day the animals were trained on access to the enriched cage and every other second day on access to the barren cage. The animals could hear the humming sound (the conditioned stimulus, CS) and see the test person during training of neighbouring animals. Minks were trained every weekday (18 days in total) with no interval between CS and US during the first week of training. During the last three weeks of training the interval CS – US was gradually extended from 0 s to 2 min.

During the first 5 days the animals were trained once a day, at 09:00 h and were let back into the standard cage at 15:00 h. The second week of training, the animals were trained twice a day, at 09:00 h and again at 12:00 h. At the second training session, the animals responded very inactively or not at all (stayed in the nest box) following the CS, which could be due to the fact that they had been satisfied ethologically during the first training session (through play or physical activity in general). Furthermore a study has shown that farmed mink have their primary activity period from 04:00 to 10:00 (Hansen et al., 1994), which could possibly also have an impact on the low responsiveness. After three days of observing this low responsiveness, training was performed once a day to get a homogenous behavioural response in

the interval CS – US. During the last training sessions it was noted that 14 of the 16 subjects showed a high level of activity (high number of behavioural transitions) following CS, which indicated a successful conditioning. The slightly different behaviour of the last two animals was considered an expression of natural variation, and all animals were included in the anticipation test. Before the anticipation test started in January 2006, all animals were habituated to the technical procedures in relation to video recording in order to minimize effects of external disturbances.

- test procedure

The test was conducted every weekday in the period January 2 – February 16, 2006, starting at 09:00 h. An interval of 10 min between CS (humming sound) and US (access to either enriched or barren cage) was used for the assessment of the anticipatory response. The behaviour performed during the interval was recorded on videotape (Sony Hi-8 15 ×) for further analysis. One animal was tested on one type of US (access to enriched cage or access to barren cage) every day, and the rest of the subjects were trained as usual to maintain the association of CS and US.

Experiment 2

In this experiment the anticipatory response of mink housed in enriched environments was compared to the anticipatory response of mink housed in barren environments. The anticipatory response was induced by expectation of a positive reward (unconditioned stimulus, US: cat food served through the front door of the middle cage).

- experimental housing

Eight mink were housed in an enriched environment consisting of a double cage (standard cage + enriched cage), and 8 mink were housed in a barren environment consisting of a double cage (standard cage + barren cage without nest box). The enrichments in the enriched cage were as follows: Extra supply of straw in the nest box, 1 net cylinder attached to the ceiling of the cage, 1 plastic cylinder lying at the bottom of the cage, 1 sisal robe attached to the ceiling with a dried pig ear attached to the end, 1 floater for fishing nets, 3 plastic balls, and 1 piece of robe (10 cm) with knots. The dimensions of the two cylinders were the same as in Experiment 1 and they were regularly filled with straw. The mink were kept in their housing conditions for 12 days before training was started, to make sure that the

animals were influenced by their individual housing condition.

- conditioning procedure

All mink had to learn the association of a conditioned stimulus (CS: One click from a dog training clicker) with an oncoming reward (an unconditioned stimulus, US: Cat food, Landlord poultry flavour) by pairing in a Pavlovian paradigm. The training sessions were conducted every weekday in the period March 1 – March 31, 2006, 23 days in total. The training started at 09:00 h and ended at 12:00 h. During the first week of training there was no interval between the CS and the US. Thereafter the interval was gradually extended from 0 s to 2 min.

During the last five days of training it was noted that at least 13 of the 16 subjects showed a high number of behavioural transitions following the CS. The conditioning was therefore considered successful. Before beginning the anticipation test in April 2006, all animals were habituated to the technical procedures in relation to video recording.

- test procedure

The test was conducted in the period April 2 – April 10, 2006. Two mink were tested per day, with the first test starting at 09:00 h every day. The anticipatory response was registered during a period of 10 min from the onset of the CS to the onset the US. The interval was recorded on videotape (Sony Hi-8 15 ×) for further analysis. The rest of the experimental subjects were trained as usual to maintain the association between CS and US.

Experiment 3

The two groups of mink from Experiment 2 were interchanged between the two environmental conditions. The animals were left in these environments for 12 days (as in Experiment 2). The training of the animals was conducted in the period April 23 – May 23, 2006. The animals had already learnt the association of the CS (click) and the US (cat food) during Experiment 2, so they were trained only 13 days in total. Otherwise, the training was conducted the same way as in experiment 2. The anticipation test was carried out in the period May 25 – June 1, 2006, and consisted of exactly the same procedures as those of Experiment 2.

Data analysis and statistical methods

All recordings were analysed using the software programme The Observer, version 2.0, Noldus information Technology, Wageningen, The Netherlands. Continuous recording (Martin & Bateson, 1993) of all of the behavioural elements described in Table 1 was used as a basis of calculation of the total number of behavioural transitions (the anticipatory response). In experiment 1, all animals were their own controls and were tested for differences in number of behavioural transitions as matched pairs, using The Wilcoxon Signed Ranks Test (one-tailed) (Siegel & Castellan, 1988). In Experiment 2 and 3, the two groups of mink were tested for differences in the number of behavioural transitions, using The Mann-Whitney U Test (one-tailed) (Siegel & Castellan, 1988).

Table 1. Ethogram of the observed behavioural elements during the interval CS – US in Experiment 1, 2, and 3. Indication is given if an element applies to just one or two of the experiments.

Behaviour	Description
In / out nest box	The mink went in and out of the nest box. The stay in the nest box lasted for less than 3 s.
Actively curious	The mink moved in an upright position along the walls of one of the cages or along the cage ends, sniffing and exploring the surroundings (including the observer).
Passively curious	The mink sat passively at the bottom of a cage or just outside a nest entrance observing its surroundings (including the observer).
Bite	The mink were biting in the cage net.
Ordinarily active	The mink moved around in one of the cages, with all four paws at the bottom of the cage, sniffing and exploring its surroundings.
Inactive	The mink was lying at the bottom of one of the cages.
In nest box	The mink was staying in a nest box for more than 3 seconds, with the whole body inside the nest box or with the body inside and the head just outside the entrance.
Scratch	The mink was scratching different places in the available cages (and on the partitions in Experiment 1).
Stereotypic behaviour	The mink was performing regularly, repeated, and morphological identical movements without any obvious function (e.g. Bildsøe et al., 1991).
Crawl	The mink was crawling on the cage walls with all four paws on the netting.
Relieve itself	The mink relieved itself at the end of one of the cages.
Lick	The mink was licking the cage net with the tongue.
Play	The mink jumped up and down and around itself in one of the cages and/or in one of the nest boxes, ran quickly back and forth in one of the cages in a playing manner, or played with/stayed in the enrichment objects (the latter only in Experiment 2 and 3).
Drink	The mink manipulated the water dispenser at the end of one of the cages.
Eat	The mink ate food from the ceiling of the standard cage.
Groom	The mink groomed and scratched its fur with teeth and paws.
Nose poke (Experiment 1)	The mink was standing in an upright position, or crawling, while pointing its nose (and touching the surface) at the cage net by the partitions, or directly at the partitions, or in the corners of the standard cage towards either the enriched cage or the barren cage (Vinke, 2004).
Through passage (Experiment 2 and 3)	The mink moved once through the passage between the central standard cage and the other cages.

Results

Experiment 1

Figure 1 (top, left) shows the total number of behavioural transitions in the interval CS – US when the mink were expecting either access to an enriched environment or access to a barren environment. The analysis describes every 30 s of the total 10 min

interval. It is seen that the anticipatory response declined throughout the period. The two housing conditions did not influence the response during the first 5 min of the interval but during the last part of the interval the number of behavioural transitions was highest in the group of mink that expected access to the enriched environment.

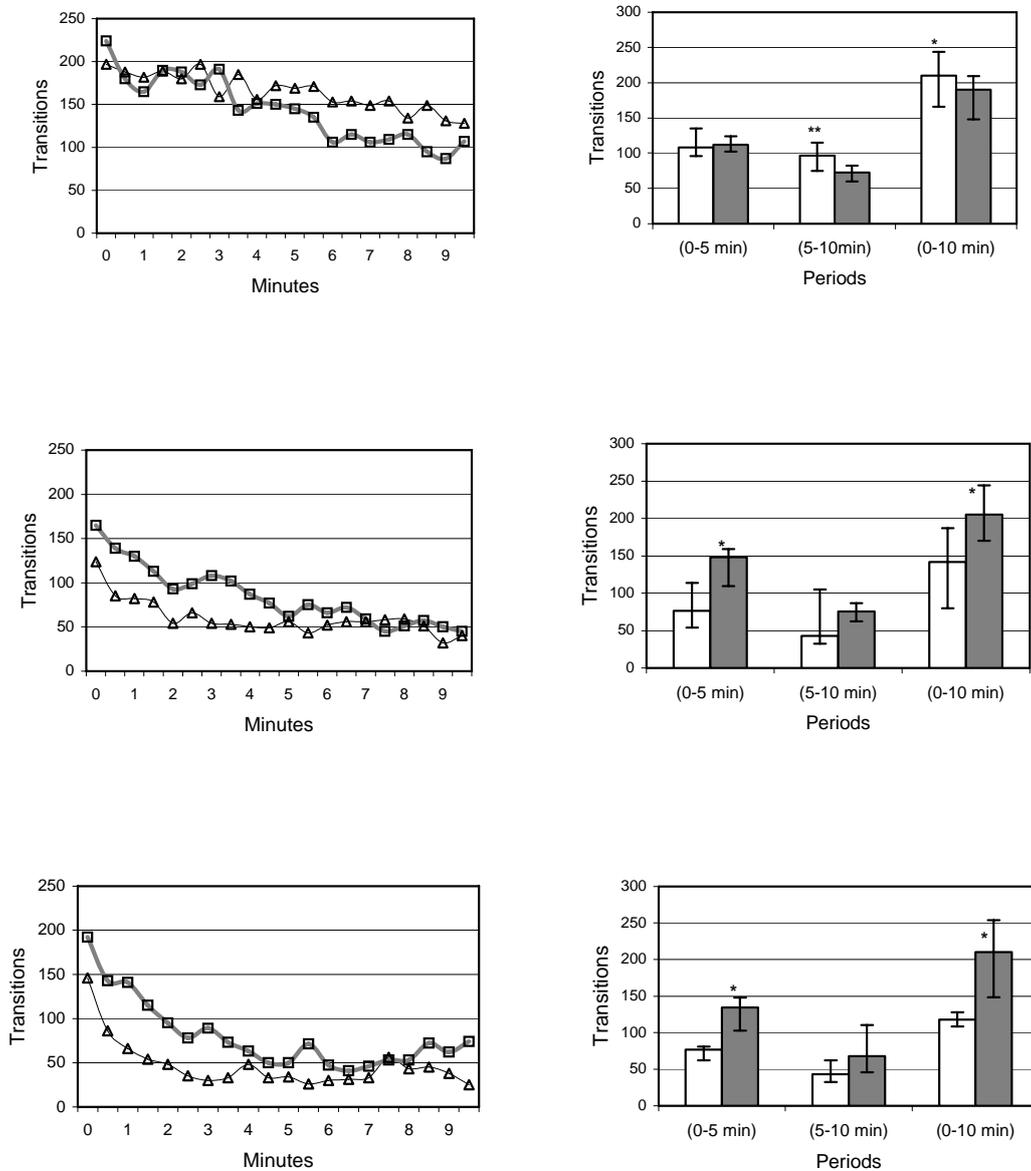


Figure 1. The total number of behavioural transitions in every 30 s of the 10 min interval CS – US (left) and mean number (+/- SEM) of behavioural transitions in the first half, in the second half, and in the whole CS – US interval (right). The top row represents Experiment 1: Squares/gray = access to barren environment, Triangles/empty = access to enriched environment. The middle and lower row represents Experiment 2 and 3, respectively: Squares/gray = barren housing condition, Triangles/empty = enriched housing condition. * $P < 0.05$; ** $P < 0.01$.

Figure 1 (top, right) shows a bar diagram of the same data as to the left depicted as the mean number of behavioural transitions (+/- SEM) in the first and in the second half of the interval, as well as in the total 10 min interval. During the last 5 min of the interval the 16 mink showed significantly more behavioural transitions in expectation of access to the enriched cage compared to the number of transitions in expectation of access to the barren cage ($z = 2.74$; $P < 0.01$). This difference was also

significant when the whole interval was analysed ($z = 2.02$; $P < 0.05$).

Experiment 2

Figure 1 (middle, left) shows the total number of behavioural transitions in the anticipation interval in the two groups of mink housed in enriched environments and barren environments respectively. The number of transitions declined during the interval for both groups. In the first 5 min of the

interval the number of transitions was highest in the group of mink housed in barren environments compared to the group of mink housed in enriched environments. The difference between the two groups diminished during the last five minutes.

Figure 1 (middle, right) shows a bar diagram of the same data as to the left depicted as the mean number of behavioural transitions (\pm SEM) in the interval CS – US for the two groups of mink. During the first 5 min as well as during the whole 10 min interval, the group of mink housed in barren environments showed significantly more behavioural transitions compared to mink housed in enriched environments ($U = 12$; $P < 0.05$ and $U = 14$; $P < 0.05$, respectively).

3.3. Experiment 3

Figure 1 (bottom, left) shows the total number of behavioural transitions in the interval CS – US of the two groups of mink housed in enriched and barren environments respectively. As in Experiment 2, the group of mink housed in barren environments had the highest number of transitions, especially in the first half of the interval.

Figure (bottom, right) shows a bar diagram of the same data as to the left depicted as the mean number of behavioural transitions (\pm SEM) in the interval CS – US for the two groups of mink. During the first 5 min. as well as during the whole 10 min. interval, the group of mink housed in barren environments showed significantly more behavioural transitions compared to the group of mink housed in enriched environments ($U = 15$; $P < 0.05$ and $U = 15$; $P < 0.05$, respectively).

Discussion

Cage enrichments, such as tubes, platforms or ropes, improve welfare in mink as measured by reduced levels of fear, abnormal behaviour and cortisol (Hansen et al., 2007; Jeppesen, 2004). Therefore, an enriched caged should be more rewarding than barren one in a Pavlovian conditioning paradigm. On this basis, the result of Experiment 1 shows that a high level of anticipatory behaviour reflects a high rewarding value of a stimulus. This result is in agreement with those of van der Harst et al. (2003a) who used the same conditioning paradigm and showed that different rewards elicited different anticipatory responses in laboratory rats. Interestingly, for the mink this effect of the reward on the anticipatory behaviour is not seen during the

first 5 minutes of the CS-US interval. Consequently, the length of the interval CS – US is an important factor when using anticipatory behaviour in a conditioning paradigm as a tool to assess the rewarding value of different stimuli.

The delayed effect of the different rewards on the number of transitions in experiment 1 may reflect that the mink in this experiment initially reacted the same way because they were housed the same way and for that reason had identical reward sensitivities. In agreement with that, the immediate effect of the housing conditions on the number of transitions in experiment 2 and 3 could reflect that the mink in these experiments were differently shaped by their different housing conditions and for that reason reacted differently as soon as the tests were started. The test situation seemed to be an exciting experience irrespective of the value of the rewards, so it may be assumed that the mink react both to the novelty or other general aspects of the test situation and to the specific rewarding properties of the situation. If this is so, experiment 1 suggests that the effect of the novel or general aspects is manifested mainly in the beginning of the test situation, and that the effect of the specific aspects shows up later.

The fact that the mink in experiment 1 reacted relatively more to the largest reward indicates that by expecting and attaining this specific reward the balance between positive and negative experiences is shifted in a positive direction and by that increasing the welfare of the animals for a short period. Experiment 2 and 3 examines whether the welfare benefits of more permanent access to enrichments are also reflected by the anticipatory behaviour of the animals.

Cage enrichments counteracting lack of stimulation, boredom, and lack of fulfilment of ethological needs reduces an individual's sensitivity to rewards (van der Harst, 2003). This is confirmed by the present results showing that mink housed in enriched environments performed less behavioural transitions compared to mink housed in barren environments. The results indicate that anticipatory behaviour may be used as a measure of the effect of enrichment on the reward-sensitivity and thereby on the welfare of mink, since welfare is also shown to be positively influenced by enrichments when it is assessed by the more traditional measures like fear, abnormal behaviour and cortisol (Hansen et al., 2007; Jeppesen, 2004). In the study by Hansen et al. mink

were exposed to the same kind of enrichments as in the present study. Considering both studies, improvement of mink welfare seems to be related to an increased complexity of the cages (e.g. via net- or plastic cylinders) in combination with occupational materials appropriate for tearing, chewing and biting (e.g. robes, balls or other objects).

The results of the present study disagrees with those obtained by Vinke et al. (2004) who could not find any significant differences in the anticipatory response between three groups of mink housed in cages differing in the amount of enrichments. This discrepancy between the results is not due to differences in the experimental procedures concerning the interval from the onset of the CS to the onset the US. Van der Harst et al. (2003b) used a 10 min interval (as in this study), whereas Vinke et al. (2004) used an interval CS – US of only 4 min. However, as shown by the present study, an interval of about 5 min from the onset of the CS to the onset of the US is appropriate to represent possible differences in the anticipatory response with this type of experimental setup. This supports the conclusion of Vinke et al. (2004) that the housing conditions of their study did not differ enough to inflict different impacts on the state of the animals. If this is correct, the results imply that mink experience barren cages to be less acceptable than enriched cages and that a small amount of enrichments like tubes and platforms in standard cages may be experienced to be just as valuable as more comprehensive enrichments. This is in line with the results of Hansen et al. (2007) who found that another potential enrichment, a doubling of the cage area, did not influence mink welfare in a positive direction as assessed by their measures.

The interval from the testing in Experiment 1 to the testing in Experiment 2 was approximately 6 weeks. In that period, the two groups of mink, housed in enriched and barren cages respectively, developed an expression of different levels of behavioural transitions in expectation of cat food during the anticipation test. The only factor differing between the two groups of mink was the degree of enrichment in the housing conditions they were exposed to, thus the obtained results must depend on that difference. In agreement with van der Harst (2003), one possible explanation might be that the enrichments changed the reward-sensitivity of the animals by shifting their internal state of balance

between positive and negative experiences in a positive direction.

The interchange of the animals in Experiment 3 showed that the setting of the internal state could be reversed within the same period, and qualitative observations during the training clearly indicated that the anticipatory response in this experiment was interchanged already after 3 weeks. These fast adjustments to more enriched surroundings add to the suggestion that the level of behavioural transitions during anticipation of a reward may be used to assess the welfare of mink. However, a few factors should be taken into account. It certainly need to be investigated whether the present results depend on the novelty of the enrichments or whether access to enrichments in the long run would influence the anticipatory behaviour in the same way and thus reflect long term relationships between enrichments and welfare. It should also be considered that the training procedure as such is an enrichment (van der Harst et al., 2005). Especially for mink from barren environments this may reduce initial high levels of behavioural transitions and result in an overestimation of the real welfare level.

With the mentioned reservations the results show that behavioural transitions during anticipation of a reward reflects the rewarding properties of enrichments as well as the effect of enrichments on reward sensitivity in mink.

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Nordic Association
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NJF-Seminar 403

Autumn Meeting

Arranged by NJF Subsection
for Fur Animals.

Kolding, Denmark August 13-15, 2007



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Screening of kit mortality on mink farms in Denmark from weaning to pelting

M. Hansen, V. Weiss, T. Clausen, M. Lassén, B. Munbjerg

Preliminary investigations among black mink females in the breeding period 2005 and brown females in the breeding period 2006 on the

connection between the females body score in the winter period, her weight development and the breeding results were carried out.

Females getting too fat up to birth get fewer live borne kits than females in normal body score. Females that are very fat in November, requires very restrictive feeding in the winter period to be in a normal body score at breeding. After that, they

easily regain weight, and are very difficult to keep at a normal body score.

Proceedings from NJF – Seminar No. 403, 11 pp, 8 figs, 7 refs. Authors' abstract.

Mastitis in mink

A.S. Hammer, C.M. Sørensen, T.H. Jensen

This report concerns preliminary results of investigations of mastitis in Danish farm mink during the period 2005-2007. The material included 71 mink females with lost or significantly reduced litters 0-2 days post partum and 38 mink females collected from 7 farms experiencing problems with mastitis. Results of pathological and microbiological investigations indicate that a large proportion (31 %) of neonatal losses were associated with bacterial infections in the mammary glands of the female mink. Several types of bacteria, mainly *Escherichia coli* and *Staphylococcus intermedius* were identified as causes of mastitis in mink. Histopathological analyses showed that *E. coli* most often causes a peracute-acute, necrotizing mastitis, while staphylococcal mastitis typically results in milder infections and abscessation of affected glands. Mortality due to mastitis were low (<5 %) on the farms included in this study, but our investigations indicate that mastitis is an important and likely underdiagnosed cause of neonatal death in mink kits.

Proceedings from NJF – Seminar No. 403, 8 pp, 3 tables, 18 refs. Authors' abstract.

Pseudomonas pneumonia in mink in Rogaland, Norway - medical and practical aspects throughout an outbreak in 2006

G. Sanson, M. Falk

Pseudomonas (Ps.) *aeruginosa* pneumonia in mink was not reported in Norway from late eighties till 2002, more than 15 years.

At Christmastime 2005, seven farms in Rogaland got Ps. *aeruginosa* infections nearly simultaneously. Infected mink were treated with

sulfadiazin/trimethoprim (Tribrissen® vet. pulver til fisk (fishpowder)). Five farms got increased mortality rate again after treatment. In these five farms a new Tribrissen®-cure was initiated combined with vaccination with a commercial vaccine (Febrivac® 3 Plus.) In one farm (farm1) Tribrissen® did no longer reduce mortality and was substituted with Terramycin® (oxytetracyclin) to reduce the infection pressure during and shortly after vaccination. Terramycin had good effect, but Farm1 lost more than 25% of the mink in one house (600 out of 2000 minks) during January.

In early July 2006 farm1 got a new outbreak of *Pseudomonas pneumonia*. Due to the experience at Christmastime, treatment with Terramycin® was initiated. This time Terramycin® didn't work and was quickly replaced with Tribrissen® - with very good effect. Controlling mortality with Tribrissen®, the farmer started to vaccinate. Unfortunately – there seemed to be no effect of the commercial vaccine (Febrivac® 3 Plus). At the 20th of July another farm (farm2) got an outbreak of *Pseudomonas pneumonia*. This farm also had the infection at wintertime and therefore already vaccinated mink-kits using Febrivac® 3 Plus. Again a total failure of the commercial vaccine, and Tribrissen® was the only way to control mortality. A third farm (farm3) got an outbreak of *Pseudomonas pneumonia* at the beginning of August. Farm3 had no outbreak at wintertime and therefore yet not vaccinated against the bacteria. Due to the lack of effect of Febrivac® 3 Plus in farm1 and farm2, it was decided not to vaccinate farm3 with the commercial vaccine.

After the failure of the commercial vaccine, bacterial cultures isolated from mink that died from *Pseudomonas pneumonia*, were sent to the vaccine-producer IDT (Impfstoffwerk Tornau-Dessau), Germany, in early August. The idea was to have IDT producing an auto-vaccine in order to control the outbreak - a process taking at least 5 weeks.

Medication with Tribrissen® was effective till about September 10th. There was no possibility to intersperse periods without treatment to reduce the risk for the development of resistances. About September 10th the mortality rate raised dramatically, especially in farm3. The application of Terramycin instead of Tribrissen did not improve the effect. There died up to 500 mink per day in farm3 after September 15th.

In late September all three farms started vaccination with the auto-vaccine (FebriVac® *Pseudomonas*) produced by IDT, which had effect immediately. Within three to five days there was a considerable decrease in mortality. After five to seven days there was no longer mortality because of infection with *Ps. aeruginosa* on these farms. Total losses in the three farms were 18% of all mink in farm1, 15% in farm2 and 50% in farm3.

From September 13th to December 30th fourteen more farms were diagnosed with *Ps. aeruginosa*-infection. Vaccination with new vaccine was successful in all cases.

Proceedings from NJF – Seminar No. 403, 2 pp. Authors' abstract.

Long term experiment confirms blue fox's good tolerance of formic acid treated feed

I. Pölönen, N. Koskinen, J. Sepponen, T. Rekilä

Regardless of several years of extensive, controlled use of formic acid in fur animal feed in Finland, without any confirmed negative effects of animal health performance, there still exist viewpoints that doubt suitability of formic acid for fur animals. For example, formic acid has been combined with rather low breeding result of Finnish blue foxes. The purpose of this experiment was to test those doubts. Therefore, incremental levels of formic acid, from 0 to 1.2% in feed were tested for long term effects in blue foxes. The highest level of formic acid, 1.2%, was 50% higher than currently recommended maximum levels. Additionally, Furmix™ concept (combination of preservatives with sulphuric acid) was tested at one level (1.4% w/w) as well. The experiment started with weaned blue fox females and lasted for two breeding seasons till weaning. All the experimental animals, four groups, 49 females in each group continued for the second year, regardless of breeding success in the first year. Experimental feeds or slaughterhouse by-products were not exposed to spoilage pressure prior to feed manufacturing or feeding. The acids were mixed in the feed, except Furmix™ that was first applied into slaughterhouse by-products, shortly before mixing into diet. Animals were fed restrictively to avoid obesity at mating. Overall results of the experiment were very good. Animals were healthy

and the cubs grow well in all feeding groups. Breeding results, counted seven weeks after parturition per inseminated female, ranged from 4.3 – 5.0 in the first parturition and from 7.2 – 8.7 in the second. There were no statistical differences between feeding groups in breeding results, insemination or whelping dates. The results of the experiment confirm that blue foxes tolerate formic acid well, and the acid does not disturb development of puberty or reproductive physiology. Furthermore, Furmix™ together with sulphuric acid proved not to have negative effects either. It can be concluded that the currently recommended maximum formic acid concentrations in blue fox feed in Finland, 0.3-0.4% during breeding, pregnancy, lactation and early growth periods, and 0.8% from September till pelting, do not jeopardize breeding results of blue foxes.

Proceedings from NJF – Seminar No. 403, 6 pp, 4 tables, 2 refs. Authors' abstract.

Protein to mink in the gestation period

T. N. Clausen, P. Sandbøl

To investigate the need of protein and amino acids in the gestation period (April 4 to April 26), and the possible importance of the gestation feed for milk production and early kit growth, we used 6 groups each consisting of 135 brown mink females in 2006 and again in 2007. The females were fed with feed from the local feed kitchen until April 6, thereafter the protein content in 2006 was changed in 5 groups from 20 percent of metabolisable energy from protein (MEp) to 52 (respectively 20, 28, 36, 44 and 52 %). In 2007 the feed protein content was also changed in five groups from April 6 so that the MEp varied from 24 to 40 % (24, 28, 32, 36 and 40 respectively). After April 26 these females had 30 % MEp. Day 28 in the nursing period, the protein content was raised to 45 MEp to satisfy the kits requirement for protein. The control group both years was fed 52 % MEp in the whole investigation period. Only females giving birth between April 26 and May 5 was included in investigation.

We conclude that females in the gestation period April 6 to April 26, need 40 % MEp or more, with this amino acid profile and energy concentration, to get the highest number of live borne kits. 32 % MEp

or less increase the number of dead borne kits, and 20 % ME_p also reduce milk production. The number of barren females was highest (not significant) in groups feed the lowest amount of protein.

Proceedings from NJF – Seminar No. 403, 11 pp, 11 tables, 9 refs. Authors' abstract.

Arginin free diets to mink kits (*Mustela vison*)

P. Sandbøl, T. Clausen, S. Lisberg, C. Hejlesen

According to the literature a number of animals can not maintain normal function of the urea cycle when fed with arginin free diet. For dogs and cats this seem to be independent of age and in cats a low activity of a specific enzyme has been pointed to as the cause. In ferrets the animals reaction change with age. Earlier investigations with mink indicated that also the mink has a arginin requirement.

The present investigation was initiated in order to check the reaction of mink to a arginin free feed ± citrulline or ornithine from 7½ weeks of age and until either a lack of symptoms or the animals reached maturity.

No abnormal behaviour was observed at neither 7½ nor 8½ weeks of age when the mink were fed the arginin free diet.

We report plasma glucose, ASAT and ALAT plus amino acids from liver and plasma. Plasma arginin were similar in the group given the arginin free feed and the control group. 6 amino acids were significantly lower in plasma from the animals fed the arginin free feed. Amongst these were glutamic acid and proline.

The results are discussed in relation to newer literature. We conclude, that the results based on the used methods and conditions show, that 7½ and 8½ weeks old mink do not have arginin as an essential amino acid; but apparently are able to supply the urea cycle via glutamic acid and proline.

Proceedings from NJF – Seminar No. 403, 16 pp, 2 figs, 4 tables, 13 refs. Authors' abstract.

Status on individual feeding in Denmark

M. Soenderup, H. Baekgaard

The computerised individual feeding has since 2003 nearly replaced the more simple method made by hand (Soenderup, 2002 and 2003). About 400 farms with more than en million mink females have today bought one of the two Danish individual feeding systems called Individual Feeding with a Farmipilot (a handheld PDA with a scanner) and Twinca Step. Nearly all new feeding machines sold from Denmark the resent last few years, a guess would be approximately 700, are prepared for Individual Feeding.

From birth to pelting the amount of feed pr cage is determined by the length of the daily period without fed, decided by the farmer. From pelting to birth the daily amount of feed is determined of the body condition of the mink.

Since 2003 individual feeding has been used for field tests at minkfarms and for projects at minkfarms of the research farms. In the promotion of Individual feeding in praxis it has been of great value investigations done by the Faculty of Agricultural Sciences, University of Aarhus former called Danish institute of Agricultural Sciences, Research Centre Foulum and Field Tests made by Danish Fur Breeders Research Centre, Holstebro and Danish Agricultural Advisory Service National Centre | Fur Animals in cooperation. Headlines of most of the investigations are mentioned, with references.

Finally the future for individual feeding is discussed.

Proceedings from NJF – Seminar No. 403, 10 pp, 1 fig, 13 refs. Authors' abstract.

The influence of body condition on breeding results and early kit mortality

H. Baekgaard, M. Ulf Hansen, M. Soenderup

The purpose of this field investigation was to examine the connection between early kit mortality

and the body condition of the females and the feed consumption from January to birth. 12 farms with a total of 5338 first year females were included in the investigation. The females were classified by types wild type, pearl and white. The body condition of the females was scored on a 6 scale within the periods; 18 – 31 of January, 20 – 24 of February, 20 – 24 of March and as pregnant females 18 – 21 of April. Moreover, some females were evaluated immediately after birth. Dead kits were noted within the first three days after birth, whenever possible. Feed allocation was registered by means of the individual feeding system with the handhold palm pilot and divided into two periods; implantation (25 of March – 6 of April) and pregnancy (7 of April – 24 of April). We did not find any coherence between the feed consumption and number of kits in any of these periods. However, we found a good correlation between the body condition of the females and the number of both living and dead kits. The body condition of the females in February, March and April and right after birth had a significant influence on the number of new born kits. We did not find any significant influence of the female's body condition in January. Generally speaking, females that are in a medium body condition show the best results. Females that are too fat in March and April had more dead- and fewer living kits. As expected the majority of the females gain weight from February to March. Those females that stay in a similar body condition or gain 1 score in body condition, have more living kits than those who gain 2 or more grades in the score. A decrease in body condition from March to April results in fewer living kits. A decrease of 2 or more grades gives furthermore a higher number of dead kits. We conclude that a conditioning of the females in February, March and April seems to be able to help the females in having more live- and fewer dead kits per female.

Proceedings from NJF – Seminar No. 403, 12 pp, 4 tables, 12 refs. Authors' abstract.

Dietary enzyme addition can increase nutrient digestibility in fur animals

A. Skrede, Ø. Ahlstrøm

Dietary supplementation of feed enzymes is common in monogastric animals fed mainly

vegetable feed ingredients, i.e. poultry and pigs. The beneficial effects are associated with enzymatic breakdown of antinutrients like β -glucans, arabinoxylan and phytate during passage through the gastrointestinal tract. The present study comprised digestibility studies with mink fed four diets with and without a supplementary enzyme product (Porzyme 8100). The diets contained barley as the sole carbohydrate source. The barley was fed untreated, fermented with lactic acid bacteria, or subjected to γ irradiation followed by soaking and incubation. Addition of feed enzymes increased significantly digestibility of starch, crude carbohydrate, crude protein and fat. Digestibility of crude carbohydrate showed significant interaction between diet and enzyme addition, while digestibility of starch, protein and fat revealed no such interaction. Dietary enzyme supplementation increased on average starch digestibility from 75.2 to 79.8%, crude carbohydrate digestibility from 51.4 to 60.2%, protein digestibility from 85.2 to 86.4%, and fat digestibility from 92.8 to 93.7%. The highest digestibility of starch and crude carbohydrate was obtained after fermentation of the barley with a *Lactobacillus plantarum/pentosus* strain followed by enzyme addition to the diet. It is concluded that digestibility of nutrients in diets for fur animals can be improved by enzyme addition.

Proceedings from NJF – Seminar No. 403, 9 pp, 2 tables, 17 refs. Authors' abstract.

Effect of excess dietary sulfur-containing amino acids on digestibility in mink

Ø. Ahlstrøm, A. Skrede, E. Kjos

The effect of dietary supplementation SAA on nutrient digestibility in adult and 10 weeks old mink was examined. Four diets were used in the experiment; basal diet, basal diet + 0.2 % DL-Met, basal diet + 0.4 % DL-Met and basal diet + 0.4 % -DL-Met and 0.2 % L-Cys (cysteine). Generally, the results showed enhanced digestibility of main nutrients, except fat, at the two highest levels of SAA supplementation. The increased digestibility of crude protein (CP) was probably partly due to higher digestibility of the supplemented SAA than CP originating from ingredients in the basal diet. The reason for the increased digestibility of other

nutrients following SAA supplementation is not clear, but it might be due to effects on digestive enzymes or gut tissue integrity. Cys supplementation increased ash digestibility significantly, indicating that mineral absorption was improved. The digestive capacity in adult mink and 10 weeks old mink were similar, except for significantly higher fat digestibility in adults.

Proceedings from NJF – Seminar No. 403, 6 pp, 2 tables, 9 refs. Authors' abstract.

The effect of ash content in feed on the amount of faecal phosphorous of blue foxes

K. Kupsala, N. Koskinen, J. Sepponen, T. Rekilä

The aim of the study was to investigate how dietary meat bone meal (MB), meat meal (M) and feather meal (F) affect the amount of manure and the solubility of dietary ash during the growth period of farmed blue foxes. The total P content and the DM content of the manure were determined.

In both experiments, the DM, ash and P content of MB and M feeds were higher than in F feed. The amount of faeces was highest in MB treatment and lowest in M treatment. Furthermore, the DM content in faeces was highest in MB treatment in both trials. The digestibility of ash was significantly lower in MB treatment compared to especially M treatment. However, the faecal P content of M treatment was higher than in MB treatment in E1 but not in E2. The faecal P content of F treatment was considerably lower than in other treatments.

In regards to the environmental load of fur farming, it would be ideal to use raw materials in the feed that has low ash content and good digestibility. The amount of 5–8 % of feather meal used in our study fulfils the ash and P requirements of growing foxes. Therefore, our study implies that environmentally the most suitable raw material could be feather meal.

Proceedings from NJF – Seminar No. 403, 9 pp, 5 tables, 8 refs. Authors' abstract.

Management of feed conversion rate in different growth phases of mink

S.H. Møller, B.K. Hansen, V.H. Nielsen

Feed conversion rates (FC) for male+female pairs of kits have been calculated for the entire growth period from 12 to 26 weeks of age, the 'Body length growth' phase (from 12 to 17 weeks of age), the 'Fat deposition' phase (from 19 to 26 weeks of age), as well as for each of the 3 weeks periods. Relations between the periods and the potential use in feeding and management strategies have been investigated. On average the FC rate in the first three-weeks-period correlated well to the entire period ($r=0.60$) and thereafter the correlation declined. FC may be calculated from feed allowance as subtraction of feed leftovers has limited effect on the results. FC rates calculated from body weight rather than weight gain significantly affects the results and thus the ranking of the mink, as high ranks based on weight gain are systematically ranked lower based on body weight. From a management point of view it could be advantageous to select animals in September, as it would allow selection to take place in a low activity period and for applying a feeding strategy in the 'Fat deposition' phase that are less likely to induce reproductive problems related obesity.

Proceedings from NJF – Seminar No. 403, 8 pp, 1 table, 4 figs, 8 refs. Authors' abstract.

Genetics of feed conversion rate in different growth phases of mink

B. Krogh Hansen, P. Berg, V. Hunnicke Nielsen, S.H. Møller

Feed conversion is assessed for genetic variation based on feed allowance, feed consumption and feed conversion rate per male and female mink pairs in different parts of the growth period from weaning until pelting. Altogether information from 4264 animals in 2131 pairs of the brown colour type is used. Genetic variation is estimated for each trait using bivariate random regression methods, including interaction between line and production

year, and effects of day of birth, litter size and weight gain. Genetic variation for feed conversion based on analysis of feed allowance and feed consumption was found for all 3-weeks periods except when the animals are 22-24 weeks of age. The genetic variation based on feed allowance and feed consumption for female kits is decreasing during the growth period. Genetic variation of feed conversion rate is estimated at 13-15, 13-18 and 13-27 weeks of age.

Proceedings from NJF – Seminar No. 403, 9 pp, 4 tables, 13 refs. Authors' abstract.

Genotype - environment interaction in mink

V.H. Nielsen, S.H. Møller, B.K. Hansen, P. Berg

Lines selected for high November weight on ad libitum (AL) and restricted feeding (RF) and for low feed conversion on ad libitum feeding (FE) were tested on both diets after three generations of selection. The genetic correlation between November weight on ad libitum and on restricted feeding was estimated to 0.92. This indicates that November weight on the two diets is not entirely the same character. Overall, the highest average November weight was obtained by selection on ad libitum feeding.

Proceedings from NJF – Seminar No. 403, 7 pp, 2 tables, 3 figs, 6 refs. Authors' abstract.

Effects of early selection and body conditioning of young blue fox *Alopex lagopus* females on breeding result, a field study

N. Koskinen, J. Sepponen, K. Kupsala, T. Rekilä

The aim of the field study was to determine if the season, when blue fox females are selected for breeding and body conditioning have an effect on the breeding result and to clarify connection of body weight of artificially inseminated blue fox female with breeding result and the litter size.

A field study with 231 blue fox females at the beginning of the trial was carried out in a private blue fox farm 2005-2006. Young blue fox females were selected for breeding in September (Early group) or late in November (Late group).

The females in Early group were fed on a restricted breeder's diet during growing-furring season and before artificial insemination. The females in group Late were fed as ad libitum during growing-furring season and the feeding was strongly restricted after selection for breeding. Commercial fox feed was used and the feed consumption was not registered due to the practical field conditions. The females of the Early group were weighed when selected for breeding, in January and at the time of artificial insemination. The females in group Late were weighed in January, in February and also at the time of artificial insemination. Breeding result (litter size/inseminated female) was calculated when litters were 25 days old.

The season for selecting the first year blue fox breeders was important. There was statistically significant difference in the average weight of females between groups in January and at the time of artificial insemination ($p < 0.0001$). At insemination time the average weights were 8848 ± 83 g and 11052 ± 158 g in groups Early and Late, respectively. The breeding result was 5.21 cubs per inseminated female in group Early and 2.37 cubs per inseminated female in group Late ($p < 0.0001$). The selection in early autumn improved the breeding result. The weight of blue fox female at the time of artificial insemination had an effect to the litter size. The litter size was significantly lower in the weight category over 11 kg at insemination time, 1.74 cubs per inseminated female ($p < 0.05$). Only 41 % of females weight over 11 kg at the time of artificial insemination weaned their cubs. However the feeding history, dieting and body conditioning had remarkable effect on breeding result. Therefore, it can be anticipated that a proper feeding and body conditioning may be potent tools to improve the breeding result of young blue fox vixens.

Proceedings from NJF – Seminar No. 403, 7 pp, 3 tables, 5 refs. Authors' abstract.

Anticipatory behaviour as a measure of welfare in mink

A. Vestergaard, A. Geisler, L. Lau Jeppesen

Mink were trained to associate a sound signal with an approaching reward. When the association was learned, the mink increased their activity as soon as they heard the sound. This anticipatory activity was quantified as number of transitions between different behavioural elements. The number of behavioural transitions was higher when the mink anticipated a reward that was known to be valuable for mink as compared to a less valuable reward, and it was also influenced by the living conditions of the mink. Mink from enriched environments performed fewer behavioural transitions than mink from barren environments in response to a standard enrichment. The possibility of using the anticipatory activity as a measure of welfare is discussed.

Proceedings from NJF – Seminar No. 403, 8 pp, 2 tables, 12 refs. Authors' abstract.

Effect of divergent selection for stereotypic behaviour in mink

L. Lau Jeppesen

Mink were selected for 5 generations for or against stereotypic behaviour. The heritability of the stereotypic behaviour was 0.26. A number of parameters were measured in the last three generations. The results showed that mink from the high stereotyping line had higher base levels as well as response levels of faecal cortisol metabolites than mink from the low stereotyping line and that more mink from the high stereotyping line than from the low stereotyping line were confident towards humans; that is, the low stereotyping line included more fearful mink. With respect to the welfare of the mink these results are contradictory. Mink from the high stereotyping line weaned more cubs and they had a lower weight in the autumn. The lower weight in the high stereotyping line may depend on the higher activity, and a persistent tendency to stay slim may explain the better cub result in this line.

Proceedings from NJF – Seminar No. 403, 6 pp, 5 tables, 7 refs. Author's abstract.

Effects of climbing cages and group size on behaviour and production in juvenile mink

H.M.K. Lindberg, S.W. Hansen, E. Aldén, L. Lidfors

The effects of climbing cages and group size on behaviours and bite marks were studied in 330 juvenile mink. Mink of the colour types Demi buff and half Sapphire were housed from weaning in pairs in standard cages, in pairs in climbing cages, 2 females and 1 male in climbing cages or 2 females and 2 males in climbing cages. Behaviours were observed during sun rise and sunset from July until November. In November an optical inspection for bite marks on the leather side were made. The occurrence of stereotypies was very low. Pair housed mink in standard cages showed no differences in behaviours or frequency of bite marks compared to pair housed mink in climbing cages. An increase in aggression and more bite marks as a consequence in group housing were not observed. The group size though affected behaviours. Mink in groups were more active out in the cage, used the enrichments less and spent less time in the nest box than pair housed mink. In conclusion our study showed that an increase in cage size and complexity of cage environment did not affect behaviours or frequency of bite marks in juvenile mink housed in pairs and the frequency of bite marks did not increase in group size of 3 or 4 individuals.

Proceedings from NJF – Seminar No. 403, 6 pp, 2 tables, 1 fig, 10 refs. Authors' abstract.

Pair housing in young silver fox vixens: some behavioural and reproductive consequences

A.L. Hovland, C. Hansen, H.C. Svensson, M. Bakken

The aim of the study was to examine behavioural and reproductive consequences of pair housing in young silver fox vixens in relation to solitary housing and to different cage environments. Sixty young vixens were separated in four experimental groups: three groups in where sisters were pair housed in double wire mesh cages and one group with solitary housed vixens. The pair housing study was completed from September to December. To investigate potential consequences of different cage

environments on social behaviour and competition the pair housed groups were given variable access to top nest boxes and food trays. Group 1 (8 pairs) had access to two nest boxes and two food trays, Group 2 (8 pairs) had access to two nest boxes and one food tray and Group 3 (8 pairs) had access to one nest box and one food tray. The solitary vixens (N=12) were housed in single cages with access to one nest box and one food tray. All groups had access to activity objects. In December all vixens were housed singly and were, in relation to the initial heat examinations in February, moved to a larger fox house in where they were housed throughout the breeding season. Recordings of social behaviour, play and stereotypic behaviour in solitary and Group 1 foxes were conducted in November and December. The vixens' use of the cage environment was recorded in all pair housed groups (1-3) in November. A food competition test was completed to establish the dominance relation within pairs. The vixens fear towards a human observer was examined approximately three weeks subsequent to the end of the pair housing experiment. Our preliminary results showed a tendency ($P=0.082$) towards more frequent play sequences ($3.7 \pm 0.88/h$) in the pair housed vixens (Group 1) compared to the solitary vixens ($1.8 \pm 0.61/h$) due to their opportunity to engage in social play. Pacing in front of the cage wall was recorded in one singly housed vixens whereas stereotypic head twirls in relation to locomotion was recorded in 50% of the solitary foxes and in 68.8% of the pair housed foxes (Group 1). Pair housed vixens hesitated to approach the human observer the first day of the fear test, however this was not significant the subsequent days. The cage environment affected the vixens' use of the area, where vixens in Group 3 stayed less in the more barren cage. Our recordings showed that the vixens were not indifferent in regard to which of the nest boxes they would use: in the pairs with access to two nest boxes (Group 1 and 2) the vixen that used one of the nest boxes was observed less often in the other nest box. There was no effect of dominance (measured as the winner of the food competition test) on the vixens use of the cages, however the lightest vixens of the pair used one of the nest boxes significantly more than the heavier counterpart, especially in Group 1 and 3. Prior to feeding, more pairs in Group 3 were involved in aggressive interactions, and there was a higher level of gaping signals in Group 3 compared to Group 2 in the period subsequent to feeding

($P=0.042$). Besides this there was no difference in total aggressive interactions ($3.8 \pm 1.18/2h$, $P>0.18$) or grooming bouts ($1.8 \pm 0.4/2h$; $P>0.18$) between the different pair housed groups. Number of mated vixens differed between groups and in average 68% of the vixens was successfully mated. The reproduction results in this study showed that only half of the mated vixens had litters three days after birth. Forty-three percent of these litters originated from Group 2. To what extent these results reflect treatment effects are at the moment difficult to tell. Further analyses will be completed to illuminate vixens' social behaviour and its reproductive implications.

Proceedings from NJF – Seminar No. 403, 12 pp, 4 tables, 3 figs, 4 refs. Authors' abstract.

Climbing cage – a practical way of housing mink?

S. Hänninen, J. Mononen, I. Pölönen, M. Miettinen

We appraised how group housing in climbing cages affects the production value of mink. The experiments were carried out on a private Finnish mink farm. The juvenile animals were housed from weaning to pelting either in male-female pairs in standard mink cages (cage 84x31x45 cm, nest box 30x31x40 cm, LxWxH) or in groups of two males and two females in climbing cages that consisted of a standard mink cage with a second floor (56x31x45 cm). There were three colour types in the experiment: black (growing season 2003), wild (2003 and 2004) and mahogany (2003). The results are based on the data from altogether 1083 animals. The housing system had no effects on the skin length, fur quality and skin price, except that the mahogany male's fur quality was slightly worse in the climbing cages than in the standard cages. The mink farmer's experiences of climbing cages were positive in terms of both animal care and their productivity. From farmers' point of view the climbing cages seem to be a feasible way to produce mink. However, since in particular aggression may be a problem in group housed mink, further studies with focus on the welfare of mink are still needed.

Proceedings from NJF – Seminar No. 403, 8 pp, 2 tables, 1 fig, 9 refs. Authors' abstract.

Order on fur animals: Research results and terms in relation to the welfare of mink

S.W. Hansen

The first Danish order on fur animals has entered into effect. The order is based on EU recommendations and contains regulations regarding the biological characteristics of the various fur animal species, the behavioural needs of the animals as well as directions regarding the design of cages and management procedures. This paper focuses on the regulations regarding mink production, and in relation to the subjects mentioned research results are stated. The below-mentioned elements are assumed to be of importance in relation to mink welfare:

- Farm mink are domesticated and to a considerable extent adapted to the physical production environment and the contact with humans and other mink. By continuously requiring selection for good animal welfare, the new order ensures that this development continues. Considering the temperament of the animals is possible by means of commercial breeding programmes.
- The primary activity periods of mink are related to sunrise and sunset. Farm mink spend 70-80% of their time in the nestbox, which is the same in wild mink. Thus, the behavioural need of mink for access to a nestbox is met.
- Farm mink live in accordance with their natural behaviour. They mate naturally and the female builds a nest of straw in the nestbox. They give birth only once a year, thus the number of litters cannot be increased by speeding up weaning. Weaning the kits at eight weeks of age is an optimal compromise in relation to the welfare of the female and the kits, and the litter is split up only gradually, which ensures that the animals socialise well with conspecifics as well as humans.
- The young animals are kept in pairs (male and female), which stimulates play behaviour, and they establish a rank order with male dominance. Adult mink are kept individually in accordance with their solitary lives.
- The size of the cage ensures that the mink can perform their specific behavioural elements, i.e. they can move freely, they can groom themselves, lie down, curl up to sleep, stretch their limbs, and they can withdraw to their nestbox to rest. A doubling or a quadruplication of the cage size will not improve the welfare of the mink.
- The new order requires that there is a shelf or a tube in the cage that the mink can use for resting or as a lookout. Furthermore, the female may also use the shelf or the tube as a refuge from the male or the kits. If the tube is not fixed to the sealing of the cage, it may be used as a toy that the mink may run into or move around with. These fittings contribute to improve the welfare of the mink. Studies regarding occupational materials for pulling or chewing continue to be carried out.
- The new order ensures that mink have permanent access to straw. Straw is essential in relation to nest building and is also used all the year round as an occupational material that is carried around, manipulated and chewed. Furthermore, straw is used as insulating material in the nestbox. Permanent access to straw increases the welfare of mink.
- Stereotypic behaviour is rarely seen in young animals, of which most are killed in november. Thus, stereotypic behaviour is primarily seen in winter and only in a few of the breeding animals. Like anticipatory behaviour, stereotypies are related to the time of feeding. The effects of individual feeding on stereotypies continue to be studied.
- Fur chewing is hereditary and during recent years the occurrence has been reduced considerably. New studies indicate that it is possible to reduce the occurrence of fur chewing further by means of occupational materials that the mink may pull or chew.
- The order requires that there is an empty cage between the females during the nursing period. This is to ensure peace and quietness during this sensitive period. However, in practice this also implies that the breeding females need to be moved once more.

- Mink are only handled (moved in traps) a few times during the production cycle. Handling takes place when the female is moved to the cage of the male to be mated, at weaning when the female is moved from the kits, when the litter, at the age of 9-10 weeks, is split up in pairs (male and female), when the animals are vaccinated, at fur grading, as well as when the breeding animals are moved to individual cages.
- Killing takes place at the cages and is quick and painless. Thus, transport is not necessary.
- The cage system makes it possible to monitor the animals. Thus, daily recordings of feed leftovers and faeces consistency are made. Generally the health of the animals is good and the extent and intensity of welfare problems are low.
- The order allows group housing of young mink. Group housing reduces the possibilities of monitoring the individual mink. It has not been documented that group housing has a positive effect on mink welfare. However, it has been documented that group housing increases aggression and the occurrence of bite marks.

It is concluded that the welfare of mink kept in a standard production environment is good and that the new order has improved the welfare of mink further in relation to occupational materials (permanent access to straw and shelf or tube), selection for confident temperament, limited restrictive feeding, and increase peace and quietness during the nursing period. However, not all the regulations are unambiguously positive for the welfare of mink. Moving the females in order to ensure an empty cage between each female will imply an extra strain on all the females. It has not been documented that eight days of restrictive feeding is sufficient to slim the breeding females that are often very fat. Furthermore, group housing implies an increased occurrence of bite marks compared with housing in pairs. Studies continue to be carried out in relation to improving the possibilities of occupation in mink, reducing aggression in mink kept in groups, individual feeding and the use of low-energy feed for slimming of mink without the animals feeling hungry.

Proceedings from NJF – Seminar No. 403, 2 pp. Author's abstract.

Electrocution in farmed foxes: evaluation from an animal welfare point of view

H.T. Korhonen , S. Cizinauskas, R. Viitmaa

The aim was to evaluate at what point consciousness is lost and brain activity ceases in electrically stunned blue foxes (*Alopex lagopus*), and to establish whether or not there is any return to consciousness after stunning before death. The study was conducted on 15 female blue foxes. All animals were sedated with an intramuscular injection of medetomidine. The results showed that the animal was unconscious immediately after stunning as documented by the absence of all reflexes. The EEG recording showed a status epilepticus pattern in all foxes immediately after stunning, and in none of the animals was a return to normal brain pattern observed. Such a generalised status epilepticus is connected with state of total unconsciousness and leads to ultimately to brain death. All the foxes in our experiment had respiratory arrest and heart fibrillation after stunning. The heart changes were irreversible in all cases and most probably contributed heavily to the death of the brain after stunning, as the fibrillating heart is not able to provide the necessary blood flow to the brain and other organs. This leads to failure of multiple organ systems and inevitable death. Rapid disappearance of the BAER after stunning indicates brainstem affection and death. Magnetic resonance imaging examination and histopathological examination of the brain revealed no severe changes to the brains of any of the foxes, indicating that stunning mainly affects the function of the brain without distorting the anatomy of the brain. In conclusion, electrical stunning produces an immediate and irreversible state of unconsciousness and therefore is a humane way of euthanasia of farmed foxes.

Proceedings from NJF – Seminar No. 403, 4 pp, 2 refs. Authors' abstract.

Posters

Sodium-bisulfate, ammonium chloride, benzoic and adipic acid in the feed for 28 – 56 day old mink kits

T.N. Clausen, P. Sandbøl, C. Hejlesen

The effect of ammonium chloride, Na-bisulfate, benzoic acid and adipic acid on urinary pH, in the early growth period of mink kits were tested. Further the validity of feed base excess (BE) as a predictor of urinary pH, where acids are used as additives, was evaluated.

The results showed that 0.5 % Na-bisulfate or 0.2 % ammonium chloride were the best of the investigated additives in reducing urinary pH, without reducing early kit growth significantly. 0.34 % adipic acid also reduced urinary pH, whereas 0.1 % benzoic acid had no effect compared to the control group. Urinary pH could not be predicted by calculating feed BE in this investigation.

Proceedings from NJF – Seminar No. 403, 8 pp, 5 tables, 15 refs. Authors' abstract.

Sodium-bisulfate and ammonium chloride for mink in the growing period

T.N. Clausen, P. Sandbøl, C. Hejlesen

The purpose of this investigation was to look at the long term effect of the addition to feed of 0.5 % Na-bisulfate and 0.2 % ammonium chloride on urine pH, kit growth and fur quality. In the investigation we used 3 groups of full-siblings of the colour type black consisting of each 47 male kits. The investigation was from July 7th to pelting, one group had control feed (CON), one group had control feed with the addition of 0.5 % Na-bisulfate (NABI) and the last group had control feed with the addition of 0.2 % ammonium chloride (AMM).

0.5 % Na-bisulfate and 0.2 % ammonium chloride to mink kits in the growing period had no negative effect on kit growth, pelt length and skin quality. Urine pH was measured monthly. 0.5 % Na-bisulfate reduced feed pH and reduced urine pH most of the period. 0.2 % ammonium chloride had

only a marked effect on urine pH at one measurement.

Proceedings from NJF – Seminar No. 403, 7 pp, 8 tables, 1 fig, 12 refs. Authors' abstract.

Energy distribution in mink feed from July to September

T.N. Clausen, P. Sandbøl, C. Hejlesen

The primary purpose of this investigation was to evaluate the effect of energy distribution on kit growth in July. In the investigation we used 14 groups of each 112 brown male- and female kits. The kits were fed trial feed from July 3rd to September 15th. The amount of metabolizable energy from protein (MEp) varied from 26 – 32 percent, the amount of ME from fat varied from 44 – 59 percent and carbohydrate varied from 15 – 27 percent of ME.

To achieve the best growth in July the amount of energy from protein should be at least 32 percent, the amount of energy from fat should be from 50 to 59 percent depending on protein level and carbohydrate should not exceed 18 percent of ME in this period. Within these levels we also saw the longest skins and the best fur quality, when the feeds were changed to feed kitchen feed from September 15th to pelting.

Proceedings from NJF – Seminar No. 403, 10 pp, 13 tables, 9 refs. Authors' abstract.

Investigation of health and disease in free-ranging mink in Denmark

T.H. Jensen, C.M. Sørensen, A.S. Hammer

In the period 1998-2007 we necropsied 335 free-ranging mink (*Mustela vison*) at the National Veterinary Institute, Technical University of Denmark. The majority of the mink were entrapped as part of other studies. Isotope studies have indicated that the majority of free-ranging mink may have escaped from mink farms. Escaped mink has been suggested as a potential source for

transmission of infectious diseases to wildlife or farmed mink. Thus monitoring of the health of free-ranging mink is important.

The mink were necropsied according to standard procedures. Tissue were tested for distemper virus by immunofluorescence and PCR and serum tested for antibodies against plasmacytosis by counter immunoelectrophoresis performed at the Danish Fur Breeders Laboratory. Relevant organs were cultured for bacteria including *Salmonella* and *Campylobacter*. McMaster and Baerman were applied for parasitological analysis. Body mass index was calculated as the weight [kg] divided by [the body length (m)]³.

The free-ranging mink were healthy and no evidence of infectious diseases were found. The mink were in good and stable body condition throughout the years of analysis. Blood samples from 27 mink (8 %) were positive for antibodies against plasmacytosis, but the presence of infection could not be confirmed by further pathological and virological examination. These results indicate that Danish free-ranging mink possess a minor risk for spreading of disease to farmed mink and other wildlife.

Proceedings from NJF – Seminar No. 403, 1 pp. Authors' abstract.

Virtual mapping of fur animal diseases

J. Østergaard

An internet based presentation of the actual disease situation on fur farms in Denmark is presented. It is possible for the individual farmer to log on and see the actual disease situation for his own farm and farms around him.

The map may show the status for aleutian disease as well as other contagious diseases like distemper, virus enteritis, *Pseudomonas* and interstitial pneumonia.

Proceedings from NJF – Seminar No. 403, 1 pp. Author's abstract.

Competition in silver fox vixens (*Vulpes vulpes*): Fighting behaviour and the impact of RHP

A.K. Akre, A.L. Hovland, I.L. Andersen, M. Bakken

Little is known about stability in RHP and how resource value affects competition in farm animals, and this should be emphasised when grouping farm animals. The main aim of this study was to investigate stability in RHP over several years in silver fox vixens. We also examined fighting variability between winners and losers as well as fighting intensity in relation to expected resource value in food competition tests. Forty-two 5-6 months old vixens were tested in pairs in a total of seven food competition tests and were given a score from zero to seven according to fights won. Two years later eight high score (HS) and eight low score (LS) vixens were retested in a single food competition test (HS versus LS). Prior to testing vixens were starved for 24h. During the competition test the animals were introduced to a wire mesh cage unfamiliar to both animals and food paste (equivalent to approximately 50% of their daily ration) was deposited in the middle of a food tray. The foxes' behaviour was video recorded for 30 minutes and then separated. None of the vixens were injured during the test. The results showed that RHP was consistent in seven of eight pairs (87.5%). HS vixens consumed more food in total ($P < 0.001$) and the biggest difference in consumption rate was found during the first ten minutes ($P < 0.001$). Intensity and variability of fights was highest during the first phase when food was most available. In conclusion there was stability in the probability of winning in a food competition test over years and the fighting behaviour varied according to expected resource value.

Proceedings from NJF – Seminar No. 403, 10 pp, 1 table, 6 figs, 13 refs. Authors' abstract.

Cross breeding – a tool in mink production

B. Krogh Hansen, H.H. Møller

The objective is

- to document heterosis effect in mink

- to compare alternative cross breeding systems
- to develop an advisory aid based on economic values that will balance the simultaneous use of pure breeding and cross breeding in the same farm

The traits primarily studied are litter size, kit survival and skin traits; secondarily temperament and feed utility in the growth period.

Alternative crosses are two-way, back crosses and three-way crosses. Two different farms are

involved. On DJF, Foulum research farm registrations on individual animals are used and heterosis for the traits temperament and feed utility are analysed. The other farm is the private farm of mink breeder Torben Bo Larsen, where information is collected on breeding line level. On both farms individual skin prices for the pure line and the cross lines are included.

*Proceedings from NJF – Seminar No. 403, 1 pp.
Authors' abstract.*

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