

# Novelty exploration, baseline cortisol level and fur-chewing in farm mink with different intensities of stereotypic behaviour



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## ABSTRACT

The present study aimed to examine the extent to which abnormal behaviours, stereotypic behaviour and fur-chewing, commonly used indicators of reduced welfare, are interrelated and linked to other welfare indicators in mink. Three groups were used based on behavioural observations, mink with no stereotypic behaviour (NST), mink performing stereotypic behaviour with only few repetitions (up to 4; ST1) and mink performing stereotypic behaviour with few (up to 4) and more repetitions (5 and above), in their repertoire ST2. Indicators of welfare included stereotypic behaviour observations, fur-chewing evaluation, baseline cortisol (faecal cortisol metabolites, FCM), and approach/avoidance behaviour in a novel object test and a stick test. There was no significant relation between the performance of stereotypic behaviour and fur-chewing. We found a higher level of FCM in the ST1 group with no tail-chewing behaviour and in tail-chewing mink in general, indicative of stress. However, unexpectedly tail-chewing mink were more explorative towards novel objects. The results supports that stereotypic behaviour and fur-chewing in mink are elicited by different environmental factors, suggested to be related to foraging and under-stimulation respectively.

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## 1. Introduction

Stereotypic behaviours are found in captive animals and are associated with past or present suboptimal aspects of the environment (Mason, 1991a). The frequency of stereotypic behaviour is generally used as a measure of reduced welfare (Broom and Johnson, 1993) also in mink (Damgaard et al., 2004; Mononen et al., 2012). Stereotyping mink have a higher baseline concentration of faecal cortisol metabolites (FCM) and they have an immediate increased sensitivity to stressors compared to non-stereotyping individuals (Malmkvist et al., 2011; Svendsen et al., 2007).

However, conflicting results have shown that individuals that spontaneously develop high levels of stereotypic behaviour may seem to overcome situations better than non-stereotyping individuals that have been treated the same way (Mason and Latham, 2004). Mink with high levels of stereotypic behaviour have a better reproduction (Jeppesen, 2004), they are less fearful towards humans (Hansen and Jeppesen, 2006) and recently a positive correlation between stereotyping mink and the formation of new hippocampal cells have been reported (Malmkvist et al., 2012).

In mink stereotypic behaviour follow a diurnal rhythm being most active during dawn and dusk and prior to feeding (Hansen and Møller, 2008). Established stereotyped behaviour is primarily observed when animals are fed restrictively as preparation for flushing and breeding,

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during Winter in Denmark and other countries on the Northern hemisphere (Damgaard et al., 2004). During prolonged periods of restrictive feeding, proto-stereotypic running may increasingly be replaced by more fixed stereotypic behaviour patterns (i.e. pacing). Feeding motivation has been shown to have an effect on stereotypic behaviour and restrictive feeding is known to provoke stereotypic behaviour in farmed mink and may thus be used as a feasible procedure in farm mink to quantify the level of established stereotypic behaviour and proto-stereotypies (Hansen and Møller, 2008). Fully developed stereotypies may be hard to determine; once fully developed stereotyped behaviour may be performed under conditions where well-being is not compromised (Mason, 1991b).

Fur-chewing is used as an indicator of reduced welfare in mink (Mononen et al., 2012). Fur-chewing is the chewing/sucking of the fur, without penetrating the skin, resulting in damaging of the hairs (Malmkvist and Hansen, 1997). The most frequent form of fur-chewing is on the tail (Hansen et al., 1998; Hansen and Møller, 2008). Fur-chewing appear functionless – like the performance of stereotypic behaviour – but may be performed in a more flexible action pattern and each bout may vary from a few seconds up to several minutes (Malmkvist and Hansen, 1997). It is primarily performed during dawn and dusk (Hansen et al., 1994), a period where mink are most active apart from prior to feeding. The performance of fur-chewing has been linked to under-stimulation due to its diurnal rhythm and pattern of occurrence during the production season (Malmkvist and Hansen, 1997). Similar types of fur-directed abnormal behaviour have been reported in other species, e.g. fur-chewing in chinchilla (Ponzio et al., 2007, 2012) and rabbits (Lidfors, 1997).

Stereotypic behaviour and fur-chewing are abnormal behaviours that both contain a genetic component in the predisposition of mink to perform them (stereotypic behaviour: Jeppesen et al., 2004; fur-chewing: Malmkvist and Hansen, 2001) but the environment is also known to have an eliciting effect, e.g. the addition of biting ropes and tubes have been shown to reduce the occurrence of both types of abnormal behaviour (Hansen et al., 2007). Previously the link between stereotypic behaviour and fur-chewing has only been investigated to a limited degree. Thus it is unknown whether e.g. stereotyping mink are less prone to develop fur-chewing or whether the types of abnormal behaviours are concurrent.

One aim of the present study was to investigate to what extent abnormal behaviours in mink – here stereotypic behaviour and fur-chewing – are interrelated. Intuitively, we hypothesised that the effect of the two types of abnormal behaviour are additive, that increasing levels of performance of the behaviours is related to decreasing levels of welfare and that they are positively related to other indicators of reduced welfare, such as e.g. elevated levels of glucocorticoids and fearfulness. Another aim was to investigate whether groups of mink differing in intensities of stereotypic behaviour (ranging from absent, to few repetitions and to many repetitions) differ in fur-chewing, baseline cortisol level, and fearfulness.

## 2. Materials and methods

### 2.1. Animals and housing

In this study we used 200 1-year-old adult female American mink (all born late April–early May 2010) from the same brown colour line used for production. They were all housed under identical conditions, individually in standard sized cages (H: 45 cm, W: 30 cm, L: 90 cm connected to a wooden nest box, H: 20 cm, W: 28 cm, L: 23 cm) at the research farm of Aarhus University, Denmark. Water was available ad libitum from a drinking nipple and straw from the top of the nest box. The mink were fed fresh feed in amounts close to ad libitum (approx. 190 kcal/100 g) once a day at 11:00 h  $\pm$  30 min.

### 2.2. Data collection

The animals were divided into three groups based on the type of stereotypic behaviour performed during behavioural observations: (1) no stereotypic behaviour observed (NST), (2) low repetitive group, with observed max of 3–4 repetitions of a stereotypic behaviour (ST1) and (3) stereotypic behaviour, with animals performing 5 or more repetitions of a stereotypic behaviour at least once out of 106 observations (ST2). The mink in ST2 were also observed performing fewer repetitions sometimes, but ST1 had always few repetitions (below 5) per bout. Additional data – used as indicators of welfare in mink (Mononen et al., 2012) – were collected in the following order: fear/exploration (stick test), baseline cortisol level (FCM sampling), stereotypic behaviour during normal and postponed feeding (behavioural observations), tail-chewing evaluation and fear/exploration (novel object test), with at least 3 days between data collection type during a 5 week period, mid August–mid September 2011.

### 2.3. Stick test

The stick test is an approach-avoidance test widely used for testing fear towards humans (e.g. (Malmkvist and Hansen, 2002)). The mink was excluded from the nest box and tested in the wire cage. The test person put a tongue spatula through the wire netting in the front, upper part of the lid section and categorised the animals immediate reaction as: (1) explorative, if the mink sniffed the stick persistently, (2) fearful: if the mink escaped towards the back and did not touch the stick, (3) aggressive: if the mink attacked and bit the stick and (4) uncertain: if the mink showed a mix of responses and could not be placed in the three other categories. The test time was 30 s and latency to touch the stick was measured as well as minimum distance to the stick estimated to nearest cm (0–90 cm).

### 2.4. Behavioural observations

The behaviour of the animals was determined based on direct scanning observations. A total of 104 individual scanning observations were made from 08:00 h to 15:00 h on 4 consecutive days with normal feeding time and 106 on 4 consecutive days with postponed feeding time at 15:00 h

**Table 1**  
Ethogram of the behaviour elements used in the scanning observations.

Elements	Description
Active	The animal was moving in the cage, exploring, eating, drinking or performing other normal behaviours.
Inactive	The animal was lying in the cage without moving.
Nest box	Half or more of the body of the animal was in the nest box.
Stereotypic behaviour	The animal repeated fixed movements at least 5 times in exactly the same stereotyped way without any obvious function. All of the movement stereotypies described by Bildsøe et al. (1991) were included in the element e.g. pacing and somersaulting. Stationary movements included vertical body movements on the spot and head-twirling.
Proto-stereotypic behaviour	Stereotypies repeated less than 5 times, both movement and stationary.

the following week starting August 22, 2011. During observations the observer walked slowly and quietly along the feed gangway and observed the cage sections (one section = 6 cages) one by one. During the observation of one section the observer stood in front of the neighbouring section to minimise disturbance. Once the animals ceased to look at the observer (typically within 2 min), the behaviour of the 6 animals in the section was scanned and classified according to one of the behaviour elements described in Table 1. We present only postponed feeding values as all behaviour elements between normal and postponed feeding were highly correlated, with higher stereotypy levels during postponed feeding.

### 2.5. Novel object test

The mink were shut into the nest box 4 min prior to testing and for 4 min post testing, thus no neighbouring mink was present during testing, to avoid e.g. social facilitation. A wooden cube (10 × 10 × 10 cm) was introduced as a novel object into the home cage, with a fresh cube used on each mink. The cube was placed approx. 50 cm from the entrance to the nest box, equidistance to the sides of the cage. The test began when the metal shutter was removed and the mink had free access to the cage and the novel object. The latency from the start of the test until the first time the mink was half out (at least the head and front legs) of the nest box as well as latency to make contact with the novel object was recorded with the fixed test time of 180 s.

### 2.6. Fur-chewing evaluation

Mink were assessed for fur damage to the tail induced by fur-chewing. Each individual was captured in a trap, examined and scored according to 5 categories: (1) no damage, (2) tail suckling, hair damage on the tip of the tail, (3) hair removed over minor areas of the tail (<1 cm), (4) hair removed over larger areas of the tail (>1/3) and (5) hair removed over majority of the tail (>2/3). Each evaluation lasted no longer than 3 min.

### 2.7. Faecal cortisol metabolites (FCM)

The main excretory route of cortisol metabolites in mink is via faeces (83% reported for females) which can be collected non-invasively and reflects the concentration of circulating cortisol in the blood (Malmkvist et al., 2011). Fresh samples of faeces were collected from wire nettings placed under the defecation zone of the cages 0–5 h after feeding. The samples were frozen at –20 °C until analysis. A total of 0.5 g (±0.01 g) of each well homogenised faecal sample was extracted with 5 ml methanol (80%) and concentrations of FCM were analysed in an aliquot of the supernatant with two different enzyme immunoassays; 11-oxo-aetiocholanolone and 11-β-hydroxy-aetiocholanolone (EIAs) previously described in detail in Möstl and Palme (2002) and Frigerio et al. (2004). We present only FCM concentrations measured by the 11-oxo-aetiocholanolone EIA, as both measures were highly correlated.

### 2.8. Statistical analysis

All calculations were performed using R 2.14.2 (R Core Development Team, 2012). A probability level (*P*) of 0.05 was chosen as the limit of statistical significance in all tests. The models were reduced by step-wise removal of non-significant terms (*P* > 0.10) starting with the interactions. The demand for dispersion and variance homogeneity was evaluated from plots of the final model residuals.

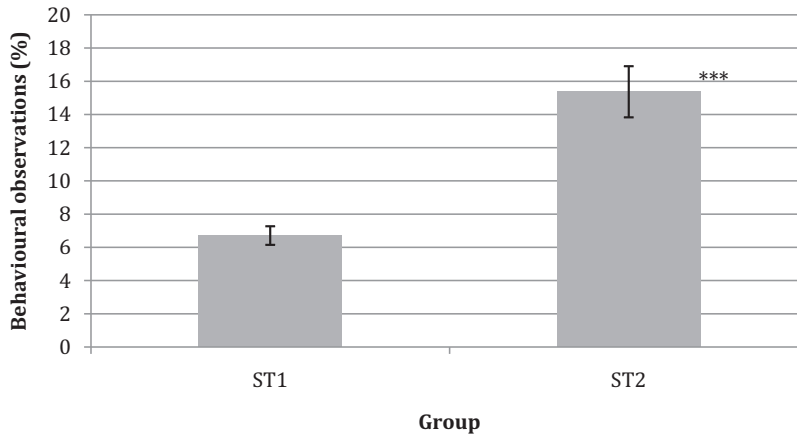
A Spearman rank-order correlation test was used to test for correlations between behaviour elements during normal and postponed feeding, between behaviours during postponed feeding and between latency to touch in the stick test and distance to stick after testing.

Differences in mean scores between stereotypy elements (movement and stationary) within groups ST1 and ST2 were tested statistically with a Mann–Whitney *U*-test and differences in the number of animals performing the stereotypy elements with a  $\chi^2$  test. Difference between groups in overall stereotypic behaviour was tested using a Student's *t*-test, as data were approximately normally distributed.

Based on the tail-chewing evaluation score the mink were divided into two groups with no tail-chewing (NC, 50.0% of animals) and tail-chewing present (TC, 50.0% of animals) (tail-chewing score 1: *n* = 100; 2: *n* = 18; 3: *n* = 21; 4: *n* = 30, 5: *n* = 31). Thus, intensity of fur-chewing is not considered in the further data analysis.

The stick-test score, being explorative or not, being fearful or not and touching the novel object in the novel object or not were analysed as a binary response in a Generalised Linear Model, including group (NST, ST1, ST2) and tail-chewing (NC, TC) as explanatory variables.

Latencies to make contact in the stick-test and novel object test were analysed using a Cox's proportional hazard (Cph) model for survival data, considering censored data (Therneau and Grambsch, 2000). The model included stereotypic group (NST, ST1, ST2) and tail-chewing (NC, TC). Mink that did not make contact within the test-period were taken as censored data.



**Fig. 1.** The mean number of observations of stereotypic behaviour ( $\pm$ S.E.) performed during postponed feeding observations for the two groups (ST1 and ST2). \*\*\* Mann–Whitney *U*-test:  $P < 0.001$ .

The concentration of FCM was log transformed and analysed in a normally distributed ANOVA including group and tail-chewing as explanatory variables. Post hoc comparisons were made using the TukeyHSD method (Crawley, 2007).

### 3. Results

#### 3.1. Behavioural observations

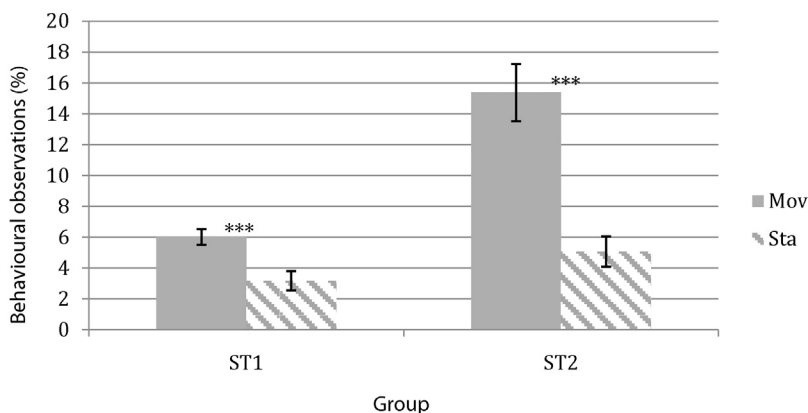
Overall, 174 mink were observed performing stereotyped behaviour during postponed feeding (ST1 always  $< 4$  repetitions,  $n = 134$ ; ST2 include  $> 4$  repetitions,  $n = 40$ ). The highest level of stereotypic behaviour was observed in group ST2, consisting of stereotyped behaviour with both high and low repetitive bouts (Fig. 1). The most prevalent stereotypy element within both groups was pacing with the highest level in ST2 (Fig. 2).

There was a positive correlation between stereotypic and inactive behaviour ( $r_s = 0.21$ ,  $P = 0.003$ ) and a negative correlation with being in the nest box ( $r_s = -0.62$ ,  $P < 0.001$ ). The inactivity and being in the nest box were negatively correlated ( $r_s = -0.62$ ,  $P < 0.001$ ), due to the time of year of

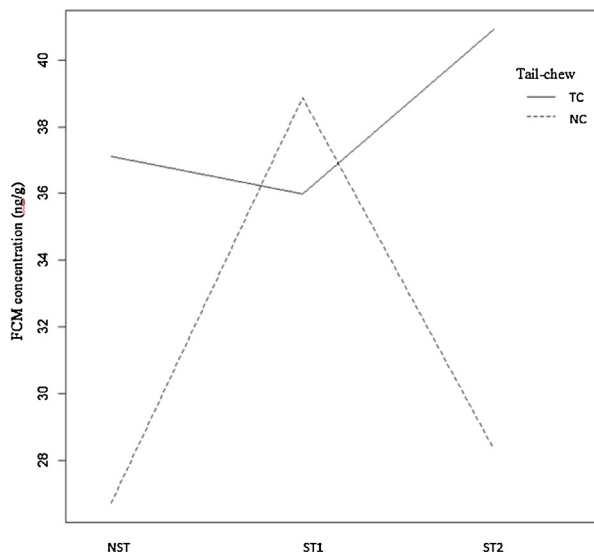
data collection, a period where mink are typically observed being inactive and resting in the cage as opposed to being in the nest box. The amount of stereotypic and other types of active behaviour did not correlate ( $r_s = 0.03$ ,  $P = 0.60$ ).

#### 3.2. Stick test

In the stick test there was no difference between either stereotypic group (NST, ST1, ST2; Explorative:  $\chi^2_{2,197} = 0.32$ ,  $P = 0.85$ ; Fearful:  $\chi^2_{2,197} = 1.85$ ,  $P = 0.40$ ) or tail-chewing (NC vs. TC; Explorative:  $\chi^2_{1,196} = 0.04$ ,  $P = 0.84$ ; Fearful:  $\chi^2_{1,196} = 0.61$ ,  $P = 0.44$ ) in the proportion of mink. Latency to touch the stick ranged from 1 s to more than the test duration (30 s). Overall, 6% of the mink never touched the stick during testing and the mink that touched had a mean latency to touch of 14 s (range 1–30 s). There was no significant difference in latency to make contact in between stereotypic groups (Cph:  $\chi^2 = 0.06$ ,  $P = 0.64$ ) or tail-chewing groups (Cph:  $\chi^2 = 4.48$ ,  $P = 0.40$ ). Most of the animals (82%) were in the front half of the cage when testing ended; thus latency to touch the stick and distance to the front part of the lid was uncorrelated ( $r_s = 0.10$ ,  $P = 0.29$ ).



**Fig. 2.** The stereotypy elements (movement and stationary) for the two groups (ST1 and ST2) are illustrated in relation to the mean number of observations in percentages ( $\pm$ S.E.) during postponed feeding. \*\*\* Mann–Whitney *U*-test:  $P < 0.001$ .



**Fig. 3.** The mean faecal cortisol metabolite (FCM) concentration (ng/g) in female mink in MONTH performing no stereotypic behaviour (NST), with only few repetitions (up to 4; ST1) and mink performing stereotypic behaviour with few (up to 4) and also more repetitions (5 and above, ST2) ( $n = 200$ ).

### 3.3. Novel object test

In the novel object test 6% of the mink never touched the novel object. There was no significant effect of group ( $\chi^2_{2,197} = 0.37$ ,  $P = 0.83$ ) on the proportion of mink that touched the novel object. There was a tendency for a higher proportion of tail-chewing mink (TC: 97%) to touch the novel object compared to mink without tail-chewing (NC: 91%;  $\chi^2_{1,196} = 3.66$ ,  $P = 0.06$ ). Latency to touch the novel object ranged from 1 to more than the test duration (180 s). TC mink that touched had a shorter latency to touch the novel object than NC mink that touched (touch: TC 26 s, 1–166; NT 43 s, 1–174; Cph:  $\chi^2 = 4.47$ ,  $P = 0.03$ ) with no effect of group of mink with different intensity of stereotypies based on repetitions (Cph:  $\chi^2 = 0.06$ ,  $P = 0.91$ ). Thus overall, tail-chewing mink appear more explorative towards the novel object than mink without tail-chewing.

### 3.4. Faecal cortisol metabolites (FCM)

There was a significant interaction between the effects of stereotypic group and tail-chewing on the FCM concentration ( $F_{2,181} = 3.51$ ,  $P = 0.03$ ). The interaction is illustrated in Fig. 3 and show that the mean FCM concentration in groups NST and ST2 were higher for tail-chewing mink opposed to a higher concentration of FCM in group ST1 when not tail-chewing.

## 4. Discussion

In the present study we found that tail-chewing mink generally had higher baseline cortisol level (FCM) concentrations compared to mink that did not tail-chew. However, one exception from this pattern was elevated FCM for non-chewing mink performing low intensity stereotypic

behaviour (ST1), defined as having 4 or less stereotypic repetitions. Additionally, tail-chewing mink were more explorative/less fearful towards novel objects. This effect could not be demonstrated towards humans in the stick test. Thus, tail-chewing mink can be interpreted as more stressed following their HPA-axis output, but less fearful. This may represent contradictive directions in relation to the traditional evaluation of welfare (e.g. Mononen et al., 2012; Mormede et al., 2007).

As expected, we found an increase in the number of animals performing stereotyped behaviour induced by postponed feeding. This indicates a relationship between high feeding motivation and the occurrence of stereotypic behaviour in accordance with previous findings (Damgaard et al., 2004; Hansen and Damgaard, 2009). Further was pacing (running) the most prevalent stereotypy element performed in this study, a behaviour that has been suggested to be associated with the motivation to forage. The postponed feeding triggered the performance of stereotypic behaviour in our study, but as stereotypies develop over time (Mason, 1991a) they are not a direct consequence of this single postponed feeding test. Rather, as suggested by Hansen et al. (2011), postponed feeding can be used as a method to evaluate the level of established fixed stereotypic behaviour including many repetitions (i.e. performed at a rate with more than 5 repetitions) and less repetitive stereotypic behaviour (i.e. performed at a rate with 3–4 repetitions) in farmed mink. We showed that low repetitive stereotypic behaviour overall was the most prevalent form of stereotypic behaviour, indicating that the stereotypic behaviour at the time of observation (early Fall) may be under development. The animals are feed in amounts close to ad libitum during early Fall. We cannot exclude that these rather low frequency stereotypies later develop into more intense stereotypies, with more repetitions later during the production cycle, e.g. during restrictive feeding prior to the yearly reproductive period next March.

Half the observed animals had some level of fur-chewing, corresponding well with fur-chewing usually being observed in adult mink post-weaning (Hansen et al., 1998). The artificial weaning of the kits at 8 weeks of age is immediate and involves complete loss of contact with the kits which combined with being rehoused and thus possibly experiencing a different feeding routine, may have a negative effect on the animals. Previous studies have demonstrated increased fur-chewing following early weaning (6 weeks) in kits (Mason, 1994). The tail-chewing individuals in our study generally had a high level of FCM, supporting that these individuals have a higher baseline level of circulating cortisol, which may be indicative of stress. Fur-chewing in mink has further been suggested to be linked with under-stimulation in the post-weaning period (Malmkvist and Hansen, 1997), and can be reduced by cage enrichment (Hansen et al., 2007). In another commercially farmed species, chinchilla, fur-chewing (unclear whether self-inflicted or as receiver) has been linked to increased HPA-axis activity and to increased fear (evaluated as lack of exploration in an elevated plus-maze test) (Ponzio et al., 2012). In our study, more of the tail-chewing animals touched the novel object and were faster at touching it in the novel object test, which could be interpreted

as an increased responsiveness. Traditionally, increased exploration has been interpreted as indicative of reduced fear, and thus positive in relation to welfare (e.g. Malmkvist and Hansen, 2002; Mononen et al., 2012). However, others have argued that motivation to seek contact with e.g. novel items also may result from under-stimulation (Meagher and Mason, 2012). In the present study both groups originated from the same breeding population and were kept under identical conditions. Thus, further studies are needed to better evaluate whether the reasons for a higher exploration really is a measure of animals being 'differently prone to under-stimulation' or whether they really have reduced fear. Until now, most data in literature (e.g. Malmkvist and Hansen, 2002; Malmkvist et al., 2003) support that object exploration in mink relates to the state of fear.

Prolonged elevated levels of glucocorticoids have a negative effect on changes in the hippocampal formation (Mcewen and Sapolsky, 1995). Low levels, however non-significant, of new hippocampal cells in tail-chewing mink have been found, compared to control and stereotyping (Malmkvist et al., 2012). Fur-chewing and stereotypic behaviour did not correlate in the present study. Thus the two types of abnormal behaviour may be triggered either by different causal factors or that animals performing less repetitive stereotyped behaviour respond to something in the environment that individuals with established stereotypies have already adapted to, illustrated by the elevated level of FCM in non-tail-chewing mink from ST1. The present results promote further detailed studies, in particular on fur-chewing behaviour, which until now, has only been studied sporadically in mink. In laboratory mice, a different mechanism behind barbering (which also lead to fur damage) and stereotypic behaviour has been suggested, although the behaviours sometimes are coined into the same term, abnormal repetitive behaviour (Garner et al., 2011). In the present study tail-chewing mink appeared to have higher FCM concentrations, reflecting increased HPA-axis-activity, which may be indicative of stress.

Further the time of year of performing various experiments or welfare evaluations on farmed mink have to be taken into account. This is consistent with a recommendation from a study investigating the development of an on-farm welfare assessment protocol for mink showing great variability in different measures throughout the production cycle (Mononen et al., 2012).

This study, performed during early fall, revealed no significant relation between levels of stereotypic behaviour and tail-chewing as well as other welfare indicators such as FCM concentrations and fear. Contrary we found tail-chewing mink to be explorative, a state normally associated with good welfare. These mink might be motivated to search for stimuli thus indicating a compromised welfare as they live in a barren environment. However, the argument may be less appropriate in the present study, as we used an identical housing environment. It could be argued that the finding relates to individual difference in mink perception. Fur-chewing and stereotypic behaviour further could partly be elicited by different environmental factors, which could be related to under-stimulation and foraging motivation respectively.

A longitudinal study investigating abnormal behaviours in mink in detail, by following individuals and using a large arsenal of welfare indicators would benefit our understanding of the link between stereotyped behaviour, fur-chewing and other welfare indicators. The finding in the present study indicates that stereotypic behaviour and fur-chewing may differ in relation to fear and FCM concentrations, traditional indicators of reduced welfare states in mink.

There was not much difference between the groups NST, ST1 and ST2, but the limit between high and low frequency stereotypic behaviour may be rather artificial. We therefore suggest that more studies into types (e.g. locomotory pacing, stationary and head-twirling) and intensity (difficult to estimate, but e.g. frequencies, time consumption and energy use) of stereotypic behaviour should be performed, as there is a large variation in performance.

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