Applied Animal Behaviour Science 203 (2018) 64–72

Contents lists available at ScienceDirect

Applied Animal Behaviour Science

journal homepage: www.elsevier.com/locate/applanim

An explorative study on the effect of provision of preferred and non-preferred enrichment on behavioural and physiological parameters in laboratory ferrets (*Mustela putorius furo*)

Marsinah L. Reijgwart[a,b,*], Claudia M. Vinke[b], Coenraad F.M. Hendriksen[a,b], Kim M. den Hoed[b], Manon I. van der Meer[b], Miriam van der Meer[a], Nico J. Schoemaker[c], Yvonne R.A. van Zeeland[c]

[a] *Institute for Translational Vaccinology (Intravacc), Antonie van Leeuwenhoeklaan 9, 3721 MA Bilthoven, The Netherlands*
[b] *Department of Animals in Science & Society, Faculty of Veterinary Medicine, Utrecht University, Yalelaan 2, 3584 CM Utrecht, The Netherlands*
[c] *Division of Zoological Medicine, Department of Clinical Sciences of Companion Animals, Faculty of Veterinary Medicine, Utrecht University, Yalelaan 108, 3584 CM Utrecht, The Netherlands*

ARTICLE INFO

Keywords:
Behaviour
Enrichment
Ferret
Physiology
Refinement
Welfare

ABSTRACT

Environmental enrichment is often advocated to refine animal studies. Despite the increasing use of ferrets as an animal model in biomedical research, the knowledge on effects of the provision of enrichment on these animals is limited. Additionally, it is unknown whether varying types of enrichment (i.e. preferred and non-preferred) have a different effect. Therefore, to explore the behavioural and physiological effects of providing (differently valued) enrichment to ferrets, three groups of six female ferrets were housed in standard conditions (with bedding, a flexible bucket, a food bowl and a water nipple), with additional non-preferred enrichment (with two ferret balls, a golf ball and an extra food bowl) and with additional preferred enrichment (with two hammocks, a foraging ball and a water bowl) for eight weeks. At the beginning and end of this period, behavioural (i.e. time spent on food and water intake, elimination, maintenance, inactivity, enrichment interaction, exploration, play, and agonistic behaviour) and physiological (i.e. bodyweight and Neutrophil/Lymphocyte ratio [N/L ratio]) parameters were recorded and compared. Results showed that agonistic behaviour increased in the ferrets housed in standard conditions, which was not observed in ferrets that were provided with preferred or non-preferred enrichment. In addition, the ferrets housed with preferred enrichment showed an increase in social play behaviour and a decrease in rearing behaviour (as part of the exploratory behaviours) which were not observed in the ferrets housed in standard conditions or with non-preferred enrichment. Moreover, the ferrets housed with preferred enrichment showed a clear preference for being inactive in the hammock and drinking from the water bowl. As there was only one cage per condition, the results of this explorative study should be considered preliminary and tentative. Nevertheless, the results are a first indication that providing laboratory ferrets with preferred enrichment has positive effects on their behaviour that are not observed in ferrets provided with non-preferred enrichment or housed in standard conditions. Therefore, we recommend to house laboratory ferrets with a hammock, foraging ball and water bowl as these enrichments might help to refine studies using ferrets.

1. Introduction

The environment of laboratory animals is closely-managed, standardized and controlled by human agency (Ohl and Putman, 2014). Often, these animals have limited living space and lack of opportunities to exert control and perform species-specific behaviour, thereby restricting the animals' ability to adapt and potentially leading to impaired health and welfare (Sambrook and Buchanan-Smith, 1997). To prevent this, the provision of environmental enrichment is advocated. However, to exert the desired positive effects on animal welfare, the enrichment must have features that are functionally and biologically relevant to the animals (Newberry, 1995). As such, sufficient knowledge of the behavioural and physiological needs of the species in question is required. For commonly used laboratory animals such as rats and mice, the requirements and effects of housing and husbandry conditions are well-documented (e.g. Bayne and Turner, 2013; Gonder and Laber, 2007). Unfortunately, for less common laboratory species, such as ferrets, this information is largely lacking. As a result, these

* Corresponding author at: Department of Animals in Science & Society, Faculty of Veterinary Medicine, Utrecht University, P.O. Box 80166, 3508 TD Utrecht, The Netherlands.
E-mail address: M.L.Reijgwart1@uu.nl (M.L. Reijgwart).

https://doi.org/10.1016/j.applanim.2018.02.014
Received 10 August 2017; Received in revised form 18 February 2018; Accepted 25 February 2018
Available online 06 March 2018
0168-1591/

animals may be housed under suboptimal conditions, which may subsequently affect the results of experimental studies (e.g. Verwer et al., 2009).

An online survey showed that pet ferrets that were confined for long periods of time, according to their owners, were less aggressive and performed more play behaviour when they were provided with more enrichment items, compared to ferrets that were given less enrichment items (Talbot et al., 2014). This study unfortunately did not specify the types of enrichments that were provided, thereby preventing specific recommendations to be made regarding the provision of enrichment to ferrets. A prior consumer demand study with laboratory ferrets determined their motivation to reach different types of enrichment (Reijgwart et al., 2016). The enrichments for which the ferrets pushed more weight than for an empty room were categorised as 'preferred' (hammock, water bowl and foraging ball). The enrichments for which the ferrets did not push more weight than for an empty room were categorised as 'non-preferred' enrichments (ferret ball and golf ball). However, it is currently unknown whether and what effects provision of (non-)preferred enrichment has on the behaviour and physiology of ferrets. This study therefore aimed to evaluate these parameters in ferrets that were housed in standard conditions, with non-preferred enrichment or with preferred enrichment for eight weeks. In mink, it was shown that access to a swimming bath (highly preferred enrichment, Mason et al., 2001) in addition to a cylinder and platform (less preferred enrichment) resulted in more play behaviour compared to provision of only the cylinder and platform (Vinke et al., 2005). It is therefore expected that preferred enrichment will have a greater (positive) effect on the behaviour and physiology in ferrets than non-preferred enrichment. Ferrets with (preferred) enrichment might show increased play behaviour and decreased agonistic behaviour, a decrease in inactivity (as an indication of less boredom- or fear-related behaviour), a decrease in exploration behaviour (as an indication of less stimulation-seeking) and a lower Neutrophil/Leukocyte ratio (as an indication of lower plasma cortisol levels), as was seen in other animals (e.g. Baumans, 2005; Broom, 1986; Davis et al., 2008; Yeates and Main, 2008).

2. Animals, material and methods

2.1. Ethical approval

This study was ethically approved by the Animal Care and Use Committee of Intravacc (DEC 201300161).

2.2. Animals

The study was performed with 18, six-month old ovariectomized female wildtype (sable) ferrets (*Mustela putorius furo*) from Marshall BioResources, USA. At the breeding facility, the ferrets were group-housed (3 ferrets per cage) in 2- or 3-tiers of mesh cages. Each cage had a dropped nesting pan with bedding in it. Prior to shipping, all ferrets were health checked and vaccinated for distemper and rabies. Upon arrival in the research facility, the ferrets were again health checked and weighed prior to being included in the study. The ferrets weighed 812 ± 83 g (min: 660 g, max: 945 g) at the start of the experiment.

2.3. Housing conditions prior to the experiment

Following arrival in the research facility, the ferrets were randomly divided over three groups of six individuals that were housed in phenolic faced plywood floor pens (1.8 m^2, 150 L × 120 w × 70 h cm) with sawdust bedding (JRS LIGNOCEL® Hygienic Animal Bedding). The room in which the ferrets were placed had an ambient temperature of 20–22 °C, a relative humidity of 50–70% and a light-dark cycle of 8:16 with artificial lighting (lights turned on at 8:00 h). Radio music was continuously played to provide auditory stimulation to the ferrets. Cages were cleaned daily and all ferrets were weighed weekly. The ferrets had ad libitum access to water (1 L drinking bottle), food (Hope Farms Ferret Balance® in a stoneware bowl) and a flexible plastic bucket that was placed on its side to provide a hiding/sleeping opportunity. This bucket was not a preferred enrichment item (Reijgwart et al., 2016) and was provided to the animals in all conditions as a shelter is regarded essential for the welfare of ferrets.

2.4. Experimental design

The ferrets were allowed to acclimatise for two weeks. At the end of the acclimatization phase, baseline behavioural and physiological measures were taken (T_0). After that, one group of ferrets remained housed in the same standard conditions (SC), whereas the other groups were provided with either non-preferred enrichment (npEC) or preferred enrichment (pEC) for a period of eight weeks (see below for further details). At the end of this period, a second series of behavioural and physiological measures was taken (T_1).

2.5. Experimental housing conditions

In the standard housing condition (SC), the ferrets had ad libitum access to water (1 L drinking bottle), food (Hope Farms Ferret Balance® in a stoneware bowl) and a flexible plastic bucket that was placed on its side to provide a hiding/sleeping opportunity. In the preferred enrichment housing condition (pEC), ferrets were provided with two hammocks, one foraging ball and one water bowl in addition to the enrichment provided in SC. In the non-preferred enrichment housing condition (npEC), ferrets were provided with two ferret balls, one golf ball and an extra food bowl in addition to the enrichment provided in SC (see Table 1 and Fig. 1 for details on the enrichment items).

2.6. Behavioural observations

At T_0 and T_1, behavioural observations were made over a 24-h period. To prevent the observer from influencing the ferrets' behaviour and enable detailed analysis of their activity pattern, infra-red surveillance cameras were installed above the enclosures to record the behaviour. Offline analysis subsequently took place using a focal-animal sampling method, recording the behaviour of each individual ferret over the entire 23-h period. To enable individual recognition of the ferrets, a different part of each ferrets' fur was shaven off (see Fig. 1). The fifteen minutes before someone entered the animal room, the thirty minutes during cleaning and feeding of the animals and the fifteen minutes after all persons had left the animal room (one hour in

Table 1
Overview of the provided enrichments in each condition. SC = standard conditions, pEC = preferred enrichment condition, npEC = non-preferred enrichment condition. pEC and npEC conditions included the enrichments from the SC condition. Numbers indicate the supplier of the enrichment. 1 = Van der Neut, Groenekan, The Netherlands, 2 = Tecnilab-BMI, Someren, The Netherlands.

Condition	Enrichment	Specifications
SC	Bucket	Flexible plastic bucket[1] on its side
	Food bowl	Adori® stoneware bowl[1] (ø18 cm, 5 cm high) filled with ad libitum Hope Farms Ferret Balance® pellets
pEC	Water bowl	Adori® stoneware bowl[1] (ø18 cm, 5 cm high) filled with tap water
	2x Hammock	Adori® hammock[1] (50 × 45 cm) attached to cage walls at 3 points
	Foraging ball	Happy Pet® tumble 'n treat[1] (ø6 cm) filled with Hope Farms Ferret Balance® pellets
npEC	Extra food bowl	Adori® stoneware bowl[1] (ø18 cm, 5 cm high) filled with Hope Farms Ferret Balance® pellets
	2x Ferret ball	Ferret ball[2] (ø25 cm) with 4 holes (ø10.2 cm)
	Golf ball	ø4 cm

Fig. 1. Pictures of the three housing conditions used in this experiment. Left: standard housing condition (SC), middle: preferred enrichment (pEC), right: non-preferred enrichment (npEC).

Table 2
Ethogram used for the observation of the ferrets.

Behaviour pattern	Contains	Description
Elimination	Urinating & defecating	Ferret backs up (often into the corner), squats with rear legs spread slightly apart, back slightly arched and tail raised over the back and urinates or defecates. Urine and faeces are not buried
Eating	Eating from bowl	Ferret takes pellet from bowl in her mouth and ingests it
	Eating from foraging ball	Ferret pushes foraging ball, causing pellets to fall out, she then takes a pellet from the sawdust in her mouth and ingests it
Drinking	Drinking from bowl	Ferret touches the water with her mouth and drinks from the water bowl
	Drinking from bottle	Ferret touches the nipple from the bottle with her mouth and drinks from the bottle
Maintenance	Scratching self	Ferret quickly moves the paw from her hind leg over her body
	Grooming	Ferret licks and gently nibbles her fur
Enrichment interaction		Ferret is in contact with the bucket, ferret ball, golf ball, hammock, foraging ball or water bowl
Inactive		Ferret sits or lies still in sawdust, bucket, ferret ball or hammock
Play	Object play	Ferret pounces, shakes, nuzzles, chews, chases, picks up or drags an object around
	Social play	Ferret runs towards or away from another ferret with a jerking, bouncing gait; biting is short and inhibited; performed with an open mouth play face; reciprocal in nature
Agonistic	Dragging	Ferret drags another ferret around while holding the neck region of the other ferret with her mouth
	Shaking	Ferret bites another ferret and holds on while shaking her head
	Neck bite	Ferret bites another ferret in the neck region
	Chase	Ferret pursues another ferret that is actively trying to move away from it
	Aggressive 'play'	Ferret performs high intensity social play behaviour, characterized by pronounced biting, little reciprocity and little bouncing
	Lunge attack	Ferret lunges towards the neck of another ferret with its mouth open
Exploration	Rearing	Ferret stands upright on her hind legs, either without support or against a wall or enrichment item
	Scratching	The ferret scratches with its front paws in the sawdust or against the wall, another ferret or enrichment object
	Tunnelling	Ferret puts its head down in sawdust and pushes forward using its hind legs, creating a tunnel in the sawdust

total, from 9:30 to 10:30) were excluded from the analysis. During the analysis, the duration of the following behaviours was recorded: elimination behaviour (urinating/defecating), maintenance behaviour (self-grooming/scratching), inactive behaviour (sleeping/lying still/resting), enrichment interaction, food and water consumption (eating/drinking), play behaviour (object and social play), agonistic interactions and exploration (rearing, scratching and tunnelling; see Table 2 for the complete ethogram).

First, a general time budget for a ferret in standard housing conditions was calculated using the average total duration for elimination, maintenance, inactivity, eating, drinking, play, agonistic and exploration behaviour from all three housing conditions at T_0. Second, for each individual ferret, the time spent on each type of behaviour at T_0 was subtracted from the time spent on this behaviour at T_1, resulting in a difference score ($\Delta T_1–T_0$) per ferret for each behaviour. For each behaviour, these difference scores were subsequently compared between the housing conditions. Third, the total durations for inactivity in the bucket, sawdust, ferret ball and hammock; eating from the bowl, sawdust and foraging ball; and drinking from the bottle and bowl at T_1 were calculated per ferret and compared between the housing conditions.

2.7. Physiological data

At T_0 and T_1, on the day following the behavioural observations, physiological measures were taken. In addition to recording the bodyweight, blood was taken for a total white blood cell count and differentiation. To facilitate blood collection the ferrets were lightly anaesthetized using medetomidine (Domitor®; 0.1 mL [T_0 = 66–95 μg/kg, T_1 = 74–113 μg/kg] IM). One hour prior to injecting the anaesthetic agent, food was removed from the enclosures to limit the risk of food regurgitation and aspiration pneumonia. Once the ferrets were sufficiently anesthetized (as evaluated by the absence of a response to toe pinching), 2 mL of blood was collected from the *vena cava cranialis* and placed in a 2 mL Vacuette® blood collection tube with EDTA as an anticoagulant. The EDTA-tubes were immediately swerved by hand, checked for blood clots and placed on a roller shaker (30 rpm) to prevent blood clot formation. If a clot was detected at this stage, another sample was taken from the ferret while

it was still anesthetized. Following collection of the blood, the ferrets were antagonised using atipamezole (Antisedan®; 0.1 mL [T_0 = 330–473 µg/kg, T_1 = 370–563 µg/kg] IM) and returned to their enclosures (where food was placed back) once they were deemed sufficiently awake. The blood samples were stored at +4 °C and a complete white blood cell count (CBC) including white blood cell differentiation (Diff) was performed within 24 h using a hematology analyser (ADVIA® 120, Perox Method). The N/L-ratio was calculated by dividing the number of neutrophils*10^3/µL blood by the number of lymphocytes*10^3/µL blood.

2.8. Statistical analysis

Statistical analyses were performed using IBM SPSS software (version 24.0). Data in the text are durations at T_0 and T_1 expressed as mean ± SD, data in the figures are differences in duration (ΔT_1–T_0) expressed as median ± IQR. Normality of distribution of the residuals was determined with an exact one-sample Kolmogorov–Smirnov test and homogeneity of variances was analysed with a Levene's test for equality of variances. Differences between the housing conditions in ΔT_1–T_0 of all parameters were analysed using a one-way ANOVA. Differences between the housing conditions and preferences within ferrets in time spent inactive in the bucket, sawdust, ferret ball and hammock; time spent eating from the bowl, sawdust and foraging bowl; and time spent drinking from the bottle and bowl at T_1 were also analysed using a one-way ANOVA. The probability level accepted for statistical significance was $p < 0.05$. The p-values were corrected for multiple comparisons using the False Discovery Rate (FDR, Benjamini et al., 2001). It should be noted that the statistical analysis ignores the shared effects of cage.

3. Results

3.1. Time budget at (T_0)

On average, the ferrets spent 3 ± 1 min eliminating, 31 ± 23 min on maintenance, 20.4 ± 0.7 h inactive, 50 ± 21 min eating, 24 ± 17 min drinking, 2 ± 2 min on play, 1 ± 1 min on agonistic behaviour, 6 ± 7 min on exploration and 1 min doing other things at T_0 (Fig. 2).

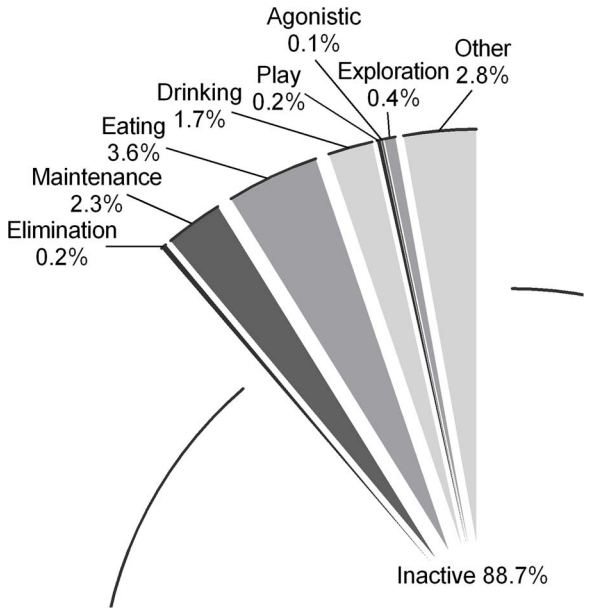

Fig. 2. The average behavioural time budget of the ferrets in this study (average % of T_0, N = 18).

3.2. Behavioural parameters

3.2.1. Elimination

The ferrets in SC, npEC and pEC showed similar changes (ΔT_0–T_1) in elimination behaviour (3 ± 1 min to 2 ± 1 min, $F_{2,15} = 0.058$, $P = 0.943$; Fig. 3a).

3.2.2. Maintenance

The ferrets in SC, npEC and pEC showed similar changes (ΔT_0–T_1) in maintenance behaviour (31 ± 23 min to 48 ± 23 min, $F_{2,15} = 1.524$, $P = 0.250$; Fig. 3b).

3.2.3. Inactivity

The ferrets in SC, npEC and pEC showed similar changes (ΔT_0–T_1) in inactivity (20.4 ± 0.7 h to 20.2 ± 0.8 h, $F_{2,15} = 0.165$, $P = 0.850$; Fig. 3c).

There were differences in the location where the ferrets spent their inactive time at T_1. The ferrets in SC, npEC and pEC spent equal amounts of time inactive in the sawdust (2 ± 3 h, $F_{2,15} = 1.430$, $P = 0.270$; Fig. 3d), but not all groups of ferrets spent equal amounts of time inactive in the bucket ($F_{2,15} = 69.158$, $P < 0.001$; Fig. 3d). More specifically, the ferrets in SC spent more inactive time in the bucket (17 ± 4 h), than the ferrets in npEC (0 ± 0 h, $p < 0.001$) and pEC (3 ± 1 h, $p < 0.001$). This is also reflected in the preferences that were observed in each group of ferrets. The ferrets in SC showed a preference for being inactive in the bucket (17.1 ± 4.4 h) over the sawdust (2.8 ± 4.4 h; $F_{1,10} = 87.314$, $P < 0.001$). The ferrets in npEC showed a preference for being inactive in the ferret ball (18.1 ± 2.0 h) over the sawdust (2.0 ± 1.7, $P < 0.001$) and the bucket (0.3 ± 0.3, $P < 0.001$)($F_{2,15} = 239.737$). The ferrets in pEC showed a preference for being inactive in the hammock (17.3 ± 2.0 h) over the bucket (2.8 ± 1.3 h, $P < 0.001$) or the sawdust (0.2 ± 0.4 h, $P < 0.001$) ($F_{2,15} = 51.666$, $P < 0.001$).

3.2.4. Enrichment interaction

The ferrets in SC, npEC and pEC showed different changes in enrichment interaction ($F_{2,15} = 47.875$, $P < 0.001$; Fig. 3e) from T_0 to T_1. More specifically, the ferrets in SC increased their time spent interacting with enrichment (3 ± 2 h to 18 ± 5 h) more than the ferrets in npEC (14 ± 0 h to 19 ± 2 h, $P < 0.001$) and pEC (20 ± 1 h to 21 ± 1 h, $P < 0.001$). Additionally, the ferrets in npEC showed a greater increase in enrichment interaction than the ferrets in pEC ($P = 0.010$).

3.2.5. Eating

The ferrets in SC, npEC and pEC showed a similar change in eating behaviour (50 ± 21 min to 37 ± 17 min, $F_{2,15} = 1.981$, $P = 0.172$; Fig. 4a).

The ferrets in SC, npEC and pEC showed equal amounts of time eating from the bowl at T_1 (26 ± 15 min, $F_{2,15} = 0.274$, $P = 0.764$, Fig. 4b). However, there was a difference between the groups in the time the ferrets spent eating food from the sawdust ($F_{2,15} = 4.508$, $P = 0.029$, Fig. 4b). More specifically, the ferrets in npEC (22 ± 20 min) spent more time eating from the sawdust than the ferrets in pEC (1 ± 2 min, $P = 0.010$). This is also reflected in the preferences that were observed. The ferrets in npEC had no preference for eating from the bowl or the sawdust (bowl: 23 ± 10 min, sawdust: 22 ± 20 min, $F_{1,10} = 0.008$, $P = 0.930$), while the ferrets in SC showed a preference for eating from the bowl over the sawdust (bowl: 26 ± 15 min, sawdust: 8 ± 6 min; $F_{1,10} = 7.662$, $P = 0.020$). The ferrets in pEC showed a preference for eating from the bowl over the sawdust and the foraging ball (bowl: 30 ± 20 min, sawdust: 1 ± 2 min; foraging ball = 2 ± 3 min $P = 0.001$ for both) ($F_{2,15} = 11.089$, $P = 0.001$).

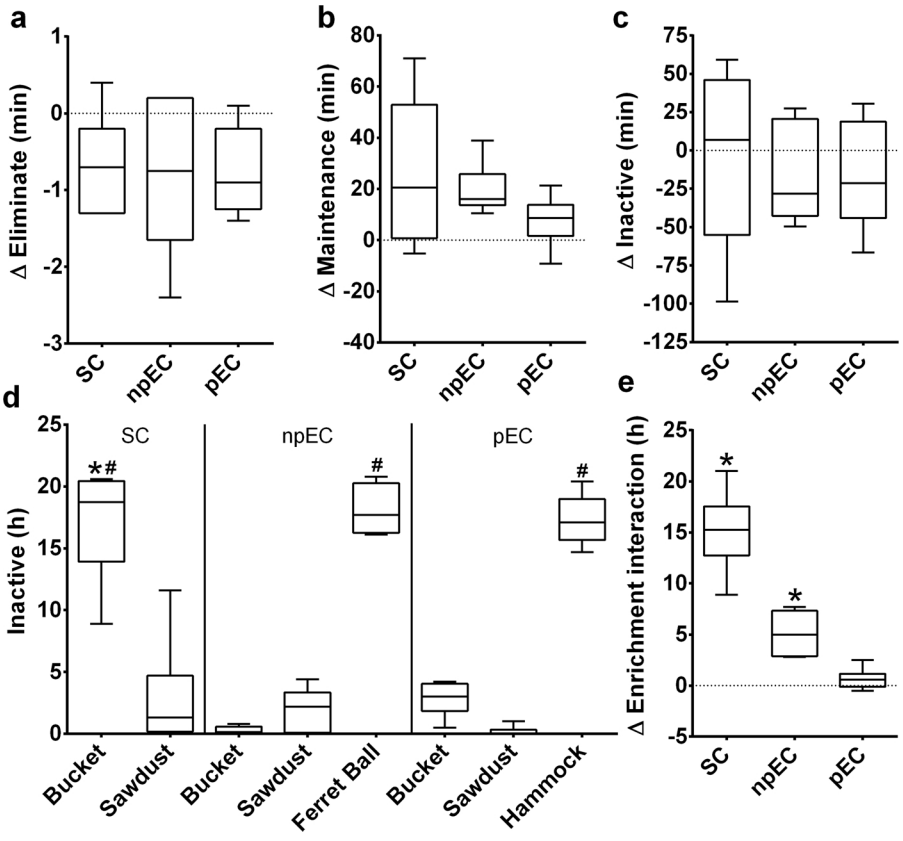

Fig. 3. The change in time the ferrets in standard conditions (SC), with non-preferred enrichment (npEC) and with preferred enrichment (pEC) spent (a) eliminating, (b) on maintenance, (c) inactive and (e) on enrichment interaction from T_0 to T_1; (d) the absolute time the ferrets spent inactive in the bucket, sawdust, ferret ball and/or hammock (median ± IQR, N = 6, * indicates a significant difference from all other groups with $p < 0.05$, # indicates a preference within a group).

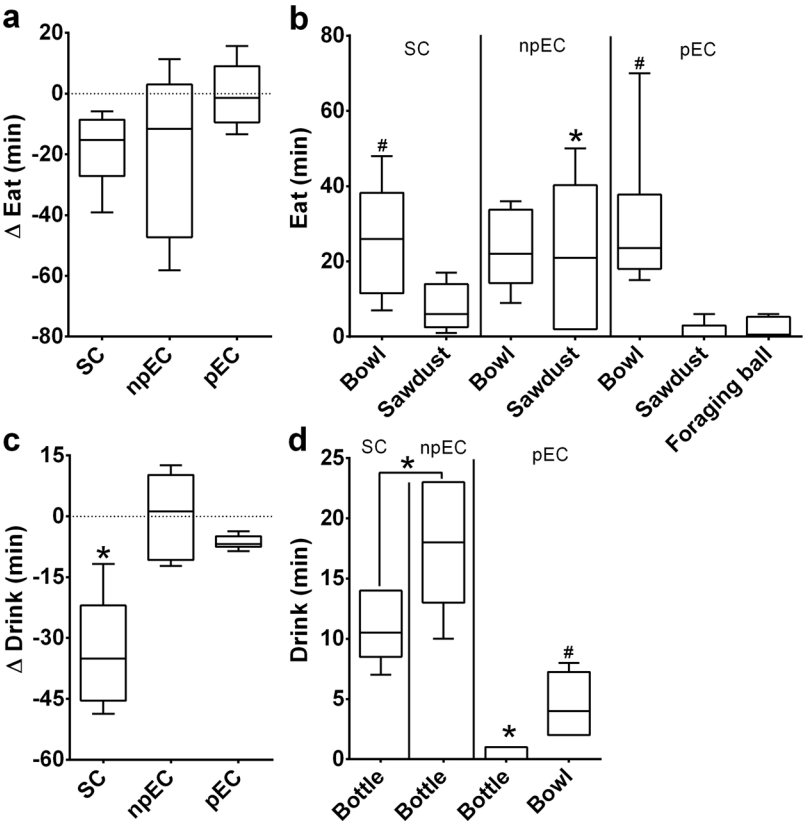

Fig. 4. The change in time the ferrets in standard conditions (SC), with non-preferred enrichment (npEC) and with preferred enrichment (pEC) spent (a) eating and (c) drinking from T_0 to T_1; the absolute time the ferrets spent (b) eating from the bowl, sawdust and foraging ball and (d) drinking from the bottle or bowl (median ± IQR, N = 6, * indicates a significant difference from all other groups with $p < 0.05$, # indicates a preference within a group).

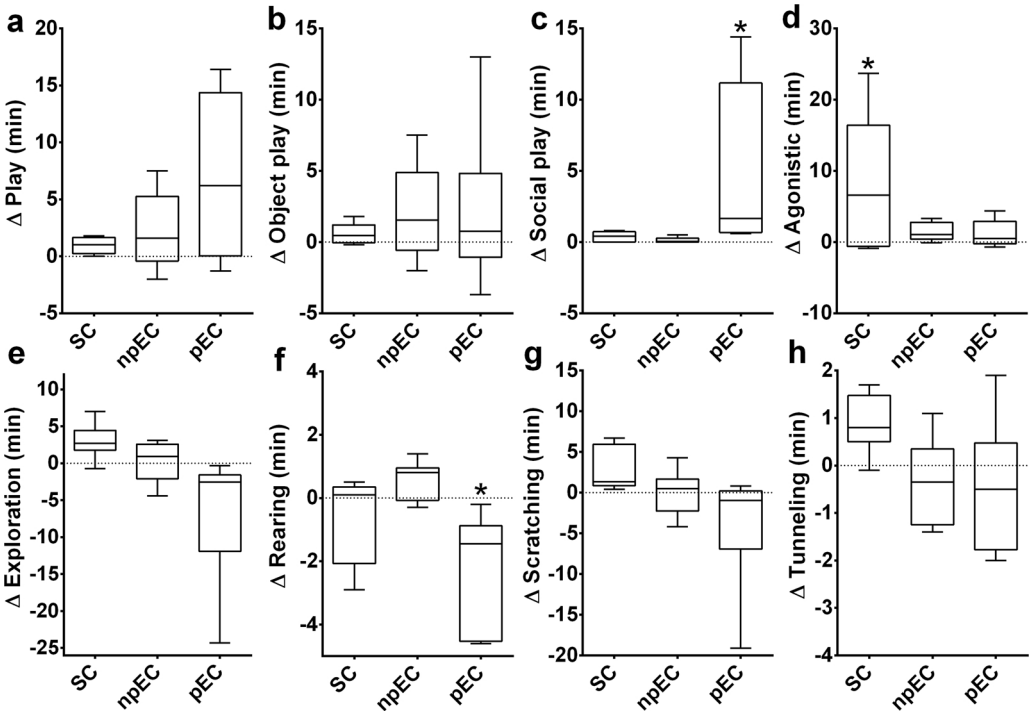

Fig. 5. The change in time the ferrets in standard conditions (SC), with non-preferred enrichment (npEC) and with preferred enrichment (pEC) spent (a) playing, (b) playing with an object, (c) on social play (d) on agonistic behaviour, (e) exploring, (f) rearing, (g) scratching, and (h) tunnelling from T_0 to T_1; (median ± IQR, N = 6, * indicates a significant difference from all other groups with $p < 0.05$).

3.2.6. Drinking

The ferrets in SC, npEC and pEC showed different changes in drinking behaviour ($F_{2,15} = 20.038$, $P < 0.001$; Fig. 4c) from T_0 to T_1. More specifically, the ferrets in SC greatly decreased their time spent drinking (44 ± 15 min to 11 ± 3 min), while the ferrets in npEC did not change their time spent on drinking (17 ± 5 min to 18 ± 5 min, $P < 0.001$). Also, the ferrets in pEC decreased their time spent on drinking less than the ferrets in SC (12 ± 3 min to 5 ± 3, $P < 0.001$).

The ferrets also spent different amounts of time drinking from the bottle at T_1 ($F_{2,15} = 40.477$, $P < 0.001$, Fig. 4d). More specifically, the ferrets in pEC (0 ± 1 min) drank less from the bottle than the ferrets in SC (11 ± 3 min, $P < 0.001$) and npEC (18 ± 5 min, $p < 0.001$). Additionally, the ferrets in SC drank less from the bottle than the ferrets in npEC ($P = 0.003$). This is also reflected in the preferences that were observed for drinking location. The ferrets in pEC preferred drinking from the bowl (5 ± 2 min) over the bottle (0 ± 1 min, $F_{1,10} = 19.322$, $P = 0.001$).

3.2.7. Play

The ferrets in SC, npEC and pEC showed similar increases in play behaviour (2 ± 2 min to 5 ± 6 min, $F_{2,15} = 2.562$, $P = 0.110$; Fig. 5a).

When play behaviour is split up in object and social play, all groups showed an equal increase in object play behaviour (2 ± 2 min to 3 ± 3 min, $F_{2,15} = 0.314$, $P = 0.735$; Fig. 5b), but showed different changes in social play behaviour ($F_{2,15} = 3.585$, $P = 0.021$; Fig. 5c). More specifically, the ferrets in pEC showed a larger increase in social play behaviour from T_0 to T_1 (0.02 ± 0.04 min to 4.87 ± 5.92 min) than the ferrets in SC (0.07 ± 0.08 min to 0.47 ± 0.32 min, $P = 0.031$) and npEC (0.00 ± 0.00 min to 0.13 ± 0.20 min, $P = 0.040$).

3.2.8. Agonistic behaviours

The ferrets in SC, npEC and pEC showed a different change in agonistic behaviour ($F_{2,15} = 3.107$, $P = 0.042$; Fig. 5d) from T_0 to T_1. More specifically, the ferrets in SC showed a larger increase in agonistic behaviour (from 2 ± 2 min to 10 ± 9 min, Fig. 5d) than the ferrets in npEC (0 ± 0 min to 1 ± 1 min, $P = 0.040$) and pEC (0 ± 0 min to 1 ± 2 min, $P = 0.044$).

3.2.9. Exploration

The ferrets in SC, npEC and pEC showed similar changes in exploration from T_0 to T_1 (6 ± 7 min to 4 ± 2 min, $F_{2,15} = 3.019$, $P = 0.079$; Fig. 5e). When exploration is split up in rearing, scratching and tunnelling, the ferrets showed different changes in rearing ($F_{2,15} = 6.085$, $P = 0.012$; Fig. 5f), but not in scratching (3 ± 6 min to 3 ± 2 min, $F_{2,15} = 2.719$, $P = 0.098$; Fig. 5g) and tunnelling (1 ± 1 min to 1 ± 1 min, $F_{2,15} = 3.180$, $P = 0.071$; Fig. 5 h). More specifically, the ferrets in pEC showed a greater decrease in rearing from T_0 to T_1 (3 ± 2 min to 0 ± 0 min) than the ferrets in npEC (0 ± 1 min to 1 ± 1 min, $P = 0.003$) and SC (1 ± 2 min to 1 ± 0 min, $P = 0.066$).

3.3. Physiological parameters

3.3.1. Weight

The ferrets in SC, npEC and pEC showed similar changes in weight (812 ± 83 g to 923 ± 78 g, $F_{2,15} = 2.327$, $P = 0.132$; Fig. 6a).

3.3.2. N/L-ratio

The ferrets in SC, npEC and pEC showed similar changes in N/L-ratio (0.54 ± 0.14 to 0.52 ± 0.16, $F_{2,15} = 2.371$, $P = 0.127$; Fig. 6b) from T_0 to T_1. At T_0.

4. Discussion

The objective of this study was to explore the effects of the provision of preferred and non-preferred enrichment items on behavioural and physiological parameters in ferrets. The ferrets that were provided with (preferred) enrichment showed different changes in play, agonistic and exploration behaviour, but showed similar or very small changes in time spent eliminating, maintenance, inactive behaviour, enrichment interaction, eating and drinking, weight and N/L ratio compared to ferrets housed in standard conditions.

The ferrets provided with preferred enrichment showed the largest increase in social play behaviour, which is regarded as a positive welfare indicator as it does not occur under stress, it acts as a reward, brings psychological benefits and is contagious (Held and Špinka, 2011;

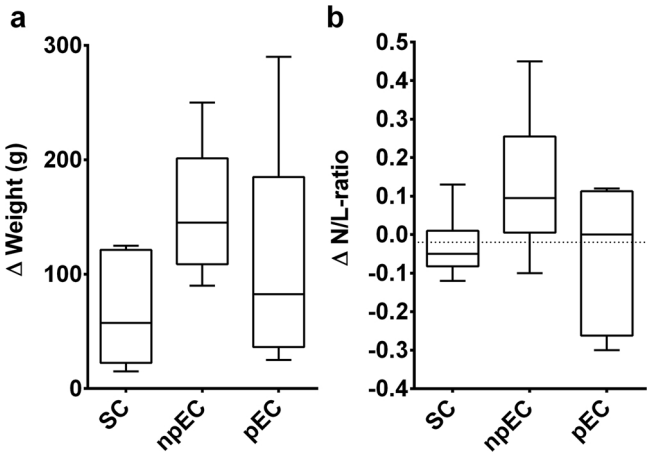

Fig. 6. The change in (a) weight and (b) N/L-ratio from T_0 to T_1 (median ± IQR) of ferrets in standard conditions (SC), with non-preferred enrichment (npEC) and with preferred enrichment (pEC) (median ± IQR, N = 6).

Oliveira et al., 2010). Our results are not in agreement with a study in mink, where the occurrence of object play behaviour was increased by the provision of cage enrichment, while the occurrence of social play did not change (Meagher et al., 2014). This difference might be explained by the presence of a manipulable object in all of the conditions for ferrets, while this was not the case for the mink. It should also be noted that the ferrets showed very little play behaviour (0.2% of the time, compared to 8% of the time – Poole, 1978), which might be an indication that even the ferrets that were provided with preferred enrichment lack stimulation to perform play behaviour. Conform expectations, the ferrets in standard conditions showed an increase in agonistic behaviour that was not observed in either enriched group. A general increase in agonistic behaviour in maturing group-housed ferrets is not surprising, as feral ferrets show intrasexual territoriality (Clapperton, 1985). Agonistic behaviour is regarded as a negative welfare indicator as it is a major cause of social stress (Blanchard et al., 2001). Our results on agonistic behaviour are in agreement with a study in mink, where the incidence of agonistic behaviour was reduced by the provision of cage enrichment (Meagher et al., 2014). The observer noticed that after eight weeks of standard housing conditions, unreciprocated play invitations often escalated in aggression in these ferrets. Potentially, the preferred enrichment reduced play frustration in the ferrets, by providing more choice in activity and/or providing an alternative outlet for frustration, which may have allowed for more positive social interaction between the ferrets. Additionally, it is possible that the enrichments provided compartmentalization of the environment, thereby reducing agonistic encounters (Desforges et al., 2016). An alternative explanation might be that the hammocks and ferret balls (both preferred resting places) allowed the ferrets to choose whether they wanted to sleep together and with whom, without having to give up their preferred resting place, possibly reducing frustration and therefore reducing aggression (Arnone and Dantzer, 1980). This could be further investigated by comparing the aggression showed by ferrets that are provided with only hammock/ferret ball versus ferrets that are provided with two hammocks/ferret balls.

Aside from changes in social interaction, the ferrets with preferred enrichment showed very little rearing behaviour at T_1, whereas this was observed more in the other two groups at this time point. This reduction in exploration time is in line with findings in rats, which reduced exploration behaviour directed at the outside of the cage when presented with enrichment in their cages (Abou-Ismail et al., 2010). As this effect was not seen in the ferrets that were given non-preferred enrichment, and the number of enrichment items were the same in for preferred and non-preferred enrichment conditions, the effects of the enrichment on rearing behaviour might be due to the value of the provided enrichment and not due to an increase in environmental complexity (Reijgwart et al., 2016). However, it should be taken into account that rearing behaviour was performed for very short periods of time and the differences between the groups were therefore also very small albeit statistically significant. This raises questions on the biological relevance of this difference in rearing behaviour between the groups. Additionally, the two other recorded exploratory behaviours (i.e. scratching and tunnelling) did not show the expected reduction after provision of preferred enrichment, as was seen in mink and rats (Dallaire et al., 2012, Abou-Ismail et al., 2010). In mink, repetitive and intensive scratching at the cage wire mesh with the front paws is a commonly observed repetitive behaviour (Hansen, 1993; Mason, 1993) and is proposed to be indicative of frustration (Mason, 1991). The behavioural motivation behind scratching and tunnelling, i.e. whether it is part of exploratory behaviour or whether it is performed as a part of play, scent-marking behaviour or as a repetitive behaviour, is unknown to the authors, possibly resulting in misclassification of these behaviours as exploratory behaviour.

The lack of difference in change in inactivity between the groups of ferrets is in agreement with a study in mink (Vinke et al., 2005). Additionally, the ferrets were as inactive as was expected based on the available values of inactivity reported in pet ferrets (Fisher, 2006). However, it should be noted that we were unable to separate time spent sleeping from time spent inactive awake, as the ferrets were not always visible, and when they were, the video footage was not detailed enough to allow us to accurately identify whether the ferrets had their eyes open (i.e. being inactive awake) or closed (i.e. sleeping). In a study in mink where inactive behaviour was studied in more detail, sleeping, the time spent inactive in the nest box (fear or anxiety induced hiding) and the time spent lying awake (a boredom-like state, respectively) were hypothesized to represent different motivations (Meagher and Mason, 2012; Meagher et al., 2013). Therefore, it is recommended to separate these three types of inactivity in future studies. Possibly, these differences in valence of inactivity can also be identified in ferrets. Possibly only one of these subtypes of inactivity was reduced in the enriched ferrets in our study. We did notice a clear preference for sleeping in the ferret ball (npEC) or hammock (pEC) over the bucket, which was expected based on the results of the consumer demand study (Reijgwart et al., 2016), possibly indicating that the standard housing conditions do not provide the ferrets with a suitable resting place.

It is not surprising that the ferrets' weight and N/L ratio reacted similarly to the ferrets' inactivity, as these parameters are closely linked (Mormède et al., 2007). For example, the HPA-axis is not only influenced by the provision of enrichment (e.g. Hansen et al., 2007), but can also be activated in response to increased activity (e.g. Girard and Garland, 2002). In turn, HPA-axis activity can affect the N/L ratio (Davis et al., 2008; Hansen and Damgaard, 1991) and the weight of an animal (e.g. Hansen et al., 2007). Ultimately, there is a great lack of consistency in the effects of enrichment on the HPA-axis and these physiological data should be viewed in light of the limitations that come with the interpretation of HPA-axis activity (Rushen, 1991). Nonetheless, it is possible that spikes in N/L ratio were missed due to the eight-week sampling interval, if the cortisol response (and, with some delay, subsequent leukocyte response) had adapted within this period, which has been suggested to occur in chickens and dogs (Hennessy et al., 2001; McFarlane and Curtis, 1989; Romero, 2004).

The ferrets' increased interaction with enrichment in standard conditions and with non-preferred enrichment were largely due to the ferrets choosing to sleep in the bucket or the ferret ball, respectively, instead of the sawdust. As a result, these changes in enrichment interaction times are unlikely to provide any information on the affective state of the animals and are probably an effect of group dynamics (i.e. the first ferret randomly choosing a resting place, where the rest joins). Likewise, no conclusions can be drawn from the observations on eating and drinking behaviour. The ferrets with non-preferred enrichment most likely ate longer from the sawdust than the other groups of ferrets

because a ferret in this group dug in the food bowl, resulting in more pellets that could be eaten from the sawdust. Similarly, the foraging ball could only hold a limited amount of pellets, making it impossible for the ferrets to forage enough food from this ball. Nonetheless, the ferrets emptied the foraging ball (which was refilled) daily. For drinking behaviour, the actual water consumption was not measured, which prevented us from determining whether the reduced drinking time of ferrets in standard conditions was a result of more efficient drinking or reduced water consumption. However, when provided a choice, as was the case for ferrets in pEC, a clear preference was observed for drinking from the bowl, while drinking from the bottle reduced to a minimum. A similar preference has been observed in mink and rabbits, possibly because a water bath/bowl allows for a more natural way of drinking (Cooper and Mason, 2000; Tschudin et al., 2011).

There were some methodological limitations to this study. Performing 24-h behavioural observations was the best method for this species, but is also very labour-intensive, therefore choices had to be made regarding the times at which the observations were made (e.g. no observations were done right after provision of the enrichment, making it impossible to assess the short-term effects) and how many days per observation point were sampled (i.e. one 24-h period per observation period is not optimal). Additionally, for each housing condition only one group of ferrets was observed due to time, physical and ethical restraints. It is preferable to observe more groups of ferrets per housing conditions as the ferrets within a group may influence each other's behaviour. Therefore, the results of this explorative study should be considered preliminary and tentative. Finally, the effects of our enrichments on the behaviour and physiology of the ferrets were not always as marked as those found in other studies and some behaviours were performed for very short periods of time, raising questions on the biological relevance of the small differences in expression for these parameters. These limitations highlight the necessity for further research to identify enrichments that can have a positive effect on behavioural and physiological parameters in laboratory ferrets. Future research should therefore focus on replicating the results found in this study, potentially also exploring the effects of other enrichments, as well as the effects of removing enrichment, group size and husbandry conditions. Additionally, other measures that might give an indication of the welfare state of the animals, such as measuring 'judgement biases' (Mendl et al., 2009) could be considered.

5. Conclusion

Overall, the ferrets that were provided with (non-)preferred enrichment showed no increase in agonistic behaviour, whereas the ferrets that were housed in standard conditions did. Additionally, the provision of preferred enrichment (hammocks, water bowl and foraging ball) to laboratory ferrets resulted in an increase in social play behaviour and a reduction in rearing behaviour, changes which were not observed in standard housing conditions or when non-preferred enrichment was provided. Since these changes have been linked to positive welfare changes in other species, provision of these preferred enrichments (hammocks, a water bowl and foraging ball) should be considered as these may help to refine studies using laboratory ferrets.

Acknowledgement

This study was funded by a grant of the Ministry of Economic Affairs (EZ) to the Institute for Translational Vaccinology (Intravacc) ("Programma coördinatiepunt alternatieven voor dierproeven").

References

Abou-Ismail, U.A., Burman, O.H., Nicol, C.J., Mendl, M., 2010. The effects of enhancing cage complexity on the behaviour and welfare of laboratory rats. Behav. Process. 85, 172–180. http://dx.doi.org/10.1016/j.beproc.2010.07.002.

Arnone, M., Dantzer, R., 1980. Does frustration induce aggression in pigs? Appl. Anim. Ethol. 6, 351–362. http://dx.doi.org/10.1016/0304-3762(80)90135-2.

Baumans, V., 2005. Science-based assessment of animal welfare: laboratory animals. Revue Scientifique Et Technique-Office International Des Epizooties 24, 503.

Bayne, K., Turner, P.V., 2013. Laboratory Animal Welfare. Academic Press.

Benjamini, Y., Drai, D., Elmer, G., Kafkafi, N., Golani, I., 2001. Controlling the false discovery rate in behavior genetics research. Behav. Brain Res. 125, 279–284. http://dx.doi.org/10.1016/S0166-4328(01)00297-2.

Blanchard, R.J., McKittrick, C.R., Blanchard, D.C., 2001. Animal models of social stress: effects on behavior and brain neurochemical systems. Physiol. Behav. 73, 261–271. http://dx.doi.org/10.1016/S0031-9384(01)00449-8.

Broom, D.M., 1986. Indicators of poor welfare. Br. Vet. J. 142, 524–526.

Clapperton, B.K., 1985. Olfactory Communication in the Ferret (*Mustela furo* L.) a Thesis Prepared in Partial Fulfilment of the Requirements for the Degree of Doctor of Philosophy in Zoology at Massey University and Its Application in Wildlife Management.

Cooper, J.J., Mason, G.J., 2000. Increasing costs of access to resources cause re-scheduling of behaviour in American mink (*Mustela vison*): implications for the assessment of behavioural priorities. Appl. Anim. Behav. Sci. 66, 135–151. http://dx.doi.org/10.1016/S0168-1591(99)00069-6.

Dallaire, J.A., Meagher, R.K., Mason, G.J., 2012. Individual differences in stereotypic behaviour predict individual differences in the nature and degree of enrichment use in caged American mink. Appl. Anim. Behav. Sci. 142, 98–108. http://dx.doi.org/10.1016/j.applanim.2012.09.012.

Davis, A., Maney, D., Maerz, J., 2008. The use of leukocyte profiles to measure stress in vertebrates: a review for ecologists. Funct. Ecol. 22, 760–772. http://dx.doi.org/10.1111/j.1365-2435.2008.01467.x.

Desforges, E.J., Moesta, A., Farnworth, M.J., 2016. Effect of a shelf-furnished screen on space utilisation and social behaviour of indoor group-housed cats (*Felis silvestris catus*). Appl. Anim. Behav. Sci. 178, 60–68. http://dx.doi.org/10.1016/j.applanim.2016.03.006.

Fisher, P., 2006. Chapter 4: ferret behavior. In: Bays, T.B., Lightfoot, T., Mayer, J. (Eds.), Exotic Pet Behavior: Birds, Reptiles, and Small Mammals. Elsevier, St. Louis, Missouri, pp. 163–205.

Girard, I., Garland, T., 2002. Plasma corticosterone response to acute and chronic voluntary exercise in female house mice. J. Appl. Physiol. 92 (4), 1553–1561. http://dx.doi.org/10.1152/japplphysiol.00465.2001.

Gonder, J.C., Laber, K., 2007. A renewed look at laboratory rodent housing and management. ILAR J. 48, 29–36. http://dx.doi.org/10.1093/ilar.48.1.29.

Hansen, S., Damgaard, B., 1991. Effect of environmental stress and immobilization on stress physiological variables in farmed mink. Behav. Process. 25, 191–204. http://dx.doi.org/10.1016/0376-6357(91)90021-Q.

Hansen, S.W., Malmkvist, J., Palme, R., Damgaard, B.M., 2007. Do double cages and access to occupational materials improve the welfare of farmed mink? Anim. Welf. 16, 63–76.

Hansen, C.B., 1993. Stereotypies in ranch mink: the effect of genes, litter size and neighbours. Behav. Process. 29, 165–177. http://dx.doi.org/10.1016/0376-6357(93)90121-7.

Held, S.D., Špinka, M., 2011. Animal play and animal welfare. Anim. Behav. 81, 891–899. http://dx.doi.org/10.1016/j.anbehav.2011.01.007.

Hennessy, M.B., Voith, V.L., Mazzei, S.J., Buttram, J., Miller, D.D., Linden, F., 2001. Behavior and cortisol levels of dogs in a public animal shelter, and an exploration of the ability of these measures to predict problem behavior after adoption. Appl. Anim. Behav. Sci. 73, 217–233. http://dx.doi.org/10.1016/S0168-1591(01)00139-3.

Mason, G.J., 1991. Stereotypies: a critical review. Anim. Behav. 41, 1015–1037. http://dx.doi.org/10.1016/S0003-3472(05)80640-2.

Mason, G.J., 1993. Age and context affect the stereotypies of caged mink. Behaviour 127, 191–229. http://dx.doi.org/10.1163/156853993x00029.

Mason, G.J., Cooper, J., Clarebrough, C., 2001. Frustrations of fur-farmed mink. Nature 410, 35–36. http://dx.doi.org/10.1038/35065157.

McFarlane, J.M., Curtis, S.E., 1989. Multiple concurrent stressors in chicks: 3. Effects on plasma corticosterone and the heterophil: lymphocyte ratio. Poult. Sci. 68, 522–527. http://dx.doi.org/10.3382/ps.0680522.

Meagher, R.K., Mason, G.J., 2012. Environmental enrichment reduces signs of boredom in caged mink. PLoS One 7, e49180. http://dx.doi.org/10.1371/journal.pone.0049180.

Meagher, R.K., Campbell, D.L., Dallaire, J.A., Díez-León, M., Palme, R., Mason, G.J., 2013. Sleeping tight or hiding in fright? The welfare implications of different subtypes of inactivity in mink. Appl. Anim. Behav. Sci. 144, 138–146. http://dx.doi.org/10.1016/j.applanim.2013.01.008.

Meagher, R.K., Dallaire, J.A., Campbell, D.L., Ross, M., Møller, S.H., Hansen, S.W., Díez-León, M., Palme, R., Mason, G.J., 2014. Benefits of a ball and chain: simple environmental enrichments improve welfare and reproductive success in farmed American mink (*Neovison vison*). PLoS One 9, e110589. http://dx.doi.org/10.1371/journal.pone.0110589.

Mendl, M., Burman, O.H., Parker, R.M., Paul, E.S., 2009. Cognitive bias as an indicator of animal emotion and welfare: emerging evidence and underlying mechanisms. Appl. Anim. Behav. Sci. 118, 161–181. http://dx.doi.org/10.1016/j.applanim.2009.02.023.

Mormède, P., Andanson, S., Aupérin, B., Beerda, B., Guémené, D., Malmkvist, J., Manteca, X., Manteuffel, G., Prunet, P., van Reenen, C.G., 2007. Exploration of the hypothalamic–pituitary–adrenal function as a tool to evaluate animal welfare. Physiol. Behav. 92, 317–339. http://dx.doi.org/10.1016/j.physbeh.2006.12.003.

Newberry, R.C., 1995. Environmental enrichment: increasing the biological relevance of captive environments. Appl. Anim. Behav. Sci. 44, 229–243. http://dx.doi.org/10.1016/0168-1591(95)00616-Z.

Ohl, F., Putman, R., 2014. Animal welfare considerations: should context matter? Jacobs

J. Vet. Sci. Res. 1, 006.
Oliveira, A.F.S., Rossi, A.O., Silva, L.F.R., Lau, M.C., Barreto, R.E., 2010. Play behaviour in nonhuman animals and the animal welfare issue. J. Ethol. 28, 1.
Poole, T.B., 1978. An analysis of social play in polecats (Mustelidae) with comments on the form and evolutionary history of the open mouth play face. Anim. Behav. 26, 36–49.
Reijgwart, M.L., Vinke, C.M., Hendriksen, C.F., van der Meer, M., Schoemaker, N.J., van Zeeland, Y.R., 2016. Ferrets' (*Mustela putorius furo*) enrichment priorities and preferences as determined in a seven-chamber consumer demand study. Appl. Anim. Behav. Sci. 180, 114–121. http://dx.doi.org/10.1016/j.applanim.2016.04.022.
Romero, L.M., 2004. Physiological stress in ecology: lessons from biomedical research. Trends Ecol. Evol. 19, 249–255. http://dx.doi.org/10.1016/j.tree.2004.03.008.
Rushen, J., 1991. Problems associated with the interpretation of physiological data in the assessment of animal welfare. Appl. Anim. Behav. Sci. 28, 381–386. http://dx.doi.org/10.1016/0168-1591(91)90170-3.
Sambrook, T., Buchanan-Smith, H., 1997. Control and complexity in novel object enrichment. Anim. Welf. 6, 207–216.
Talbot, S., Freire, R., Wassens, S., 2014. Effect of captivity and management on behaviour of the domestic ferret (*Mustela putorius furo*). Appl. Anim. Behav. Sci. 151, 94–101. http://dx.doi.org/10.1016/j.applanim.2013.11.017.
Tschudin, A., Clauss, M., Codron, D., Hatt, J.M., 2011. Preference of rabbits for drinking from open dishes versus nipple drinkers. Vet. Rec. 168, 190. http://dx.doi.org/10.1136/vr.c6150.
Verwer, C.M., van der Ark, A., van Amerongen, G., van den Bos, R., Hendriksen, C.F., 2009. Reducing variation in a rabbit vaccine safety study with particular emphasis on housing conditions and handling. Lab. Anim. 43, 155–164. http://dx.doi.org/10.1258/la.2008.007134.
Vinke, C., Van Leeuwen, J., Spruijt, B., 2005. Juvenile farmed mink (*Mustela vison*) with additional access to swimming water play more frequently than animals housed with a cylinder and platform but without swimming water. Anim. Welf. 14, 53–60.
Yeates, J.W., Main, D.C., 2008. Assessment of positive welfare: a review. Vet. J. 175, 293–300.