The effect of housing systems on the animal welfare of the European hare (Lepus europaeus).

DIPLOMARBEIT

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Einleitung


Als weiterer Indikator um das Wohlergehen der Tiere in den Gehegen zu beschreiben wurden Verhaltensparameter erfasst.

Da es nach unseren Kenntnissen auch hier in der Literatur für den europäischen Feldhasen keinerlei Referenzen bzw. keine Definitionen von speziesspezifischen Verhaltensweisen gibt, wurden Verhaltensdefinitionen für diese Spezies entwickelt, sowie verschieden Methoden zur Erfassung des artspezifischen Verhaltens miteinander verglichen.
Manuskript für die Einreichung

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Abstract

Keeping wild animals in captivity requires specific environmental conditions within their housing system to ensure an animal’s welfare and health. Only few facilities keep European hares (EBH) in captivity. Literature or recommendations concerning the husbandry of this species are lacking. Most commonly the animals are kept in traditional cage systems for domestic lagomorphs.

The aim of this study was to compare two housing systems for a population of forty captive European hares. In addition to the traditional cage systems, spacious and structured enclosures with natural materials and hiding places were built for unisex groups of four to six hares.

As an indicator of stress, faecal glucocorticoid metabolite (fGCM) concentrations were measured with an Enzyme-Immuno-Assay (EIA). Also testosterone levels were analysed in male hares by using EIA. Furthermore a descriptive behaviour study was conducted for hares in the group housing system.

Initial mean fGCM level increased up to 57.65 ng/g in mean [CI 47.05 ng/g; 70.62 ng/g] and 59.94 ng/g in mean [CI 40.52 ng/g; 88.66 ng/g] in first and second male group respectively and up to 16.19 ng/g [CI 19.76 ng/g; 13.26ng/g] and 27.93 ng/g [CI 22.88 ng/g; 34.09 ng/g] in the first and the second female group respectively after relocation into the enclosures. This initial increase of the mean fGCM level following relocation into the enclosures is regarded as ‘habituation peak’. Concentrations dropped back to mean fGCM level in cages in 3 out of 4 groups (20.31ng/g in mean [CI 12.87ng/g; 34.62ng/g]) in all male study-animals; 11.59 ng/g [CI 7.55ng/g; 15.63ng/g] in mean in members of the second female study-group within the cages. For the first female group the initial peak-values did not decrease during the test month and stayed constant around 16.19 ng/g in mean. Their original cage level was 13.19 ng/g [CI 7.55ng/g; 23.04ng/g] in mean. The second male group suddenly went back to initial high values at the end of the test month. The mean testosterone level of male hares in cages (11.22 ng/g) differed slightly, but statistically significant, to mean values of 14.41 ng/g within the enclosures.

Behaviour surveys demonstrated that exploration behaviour (50%) and locomotory activity (33.8%) were the most frequent behaviour observed. Comfort behaviour occurred with 6.3% and agonistic and socio positive behaviour was seen with 6.1% and 2.6% of all behaviours respectively.
This is the first study that evaluates housing systems for European hares. Overall the study shows that group housing has a high potential in providing an animal-friendly system for the normally solitary living hares. It provides an environment where locomotory activity and various species-specific behaviours can be performed.

Key words: European hare, housing system, welfare, health, behaviour, faecal glucocorticoid metabolites
1. Introduction

Although several studies on genetics, as well as reproductive and metabolic physiology have been conducted of the European hare (*Lepus europaeus*), there is no study that evaluates adequate housing systems for this species in captivity. Based on traditional husbandry conditions for related domestic species like rabbits, hares are mostly kept in cages. This single housing facility is space-saving, low in costs and facilitates experimental procedures due to simple identification and handling of the animals. However like Morton et al., 1993 and Baumans, 2005 already mentioned for rabbits, housing in cages hardly satisfies all behavioural needs. The environmental and space limitations hinder the full expression of the behavioural repertoire (Hansen and Berthelsen, 2000) and constrain normal locomotory activity. The restriction of movement is a severe stress factor (Morgan and Tromborg, 2007), which in chronic cases can affect the animal’s immune system (Yi Zhang et al., 2008; Martin, 2009) and reproduction rate (Sheriff et al., 2009; Moberg, 2000).

Hares are primarily regarded as a species that lives in open habitats (Santilli et al, 2004, Morton et al., 1993, Chapman and Flux, 1990) with high special and structural complexity and biodiversity (Ferretti et al, 2010, Santilli et al, 2004). Outside the breeding period the species shows a largely solitary lifestyle (Bansfield, 1974). Due to increased testosterone production at the beginning of the spring reproductive period, male hares show significant agonistic behaviour (Lincoln, 1974) involving boxing and chasing (Arnold, 2006; Corbet and Harris, 1991). This raises the question if a group housing system for European hares is adequate.

In order to compare two housing systems with a population of 40 captive European hares faecal glucocorticoid metabolite (fGCM) concentrations were gathered, using a non-invasive method that is well–established and commonly used with various domestic and non-domestic species to assess stress (Touma and Palme, 2005; Monclus et al. 2006). Furthermore behavioural patterns were evaluated and two different video analysing methods, scan sampling and continuous video recording, were compared in order to select the most suitable and reliable method for this species under group housing conditions.
2. Animals, Materials and Methods

2.1. Animals

Forty adult, healthy European hares (*Lepus europaeus*) kept at the Research Institute of Wildlife Ecology from the University of Veterinary Medicine, Vienna, were included in the study. All hares were housed in individual metabolic cages and received hare food-pellets and water ad libitum.

Twenty animals, divided into four groups (two female groups with six hares each (mean age of 43± 4 weeks and 20± 10 weeks) and two male groups with four hares each (mean age of 31±15 weeks and 28±12 weeks) were transferred to enclosures.

A control group of 20 hares (10 females and 10 males) remained within the metabolic cages. Due to potential intra-specific conflicts between male individuals during the breeding season a smaller group size for males was chosen compared to females.

All animals were ear tagged and had a subcutaneous microchip (Virbac Animal Health, Back Home Bio Tec., Woolpit UK ) for individual identification. However they could not be individually differentiated during video recording.

This study was approved by the institutional ethics committee of the University of Veterinary Medicine, Vienna in accordance with Good Scientific Practice guidelines and national legislation.

2.2. Housing Systems

Both housing systems are located in the same barn. The roof is half-covered with transparent tiles and consequently the light conditions correlate with natural daylight.

The metabolic cages (59 cm length x 50 cm height x 40 cm width) are made of wood on a steel-frame with a wire front door and a wire floor. The indoor space is divided into two compartments, the front part with a food hopper, a water bottle and a log of wood. The back part is accessible through an opening in the dividing wall.

The cages are placed on one-meter-high stands and aligned in rows. Faeces and urine are collected under the floor-grid on a removable plate.

The enclosures provide a floor space of 6m x 6m. The walls are 2.4 m high, with the first meter from ground being made of wooden boards to provide a blind for the animals. The remaining wall consists of fine meshed wire.
The floor is made of concrete except for one demarcated area of 2 x 2 meters, containing bark mulch. Inside the enclosures, wooden boxes (one per animal; 111x 73 x 64 cm) with straw bedding are provided. Additional hiding places are available under large tree trunks in the middle of the enclosure. Eight individual feeding bowls are available on three of the four sides of the enclosure to avoid competition for food. Two heated water bowls, each with a volume of 1.8 litres provide drinking water all the time.

2.3. Faecal Sampling

Faecal samples were collected from the removable plate beneath the wire grating of the cages for a period of one week prior relocation (for baseline level in the cages) and during four weeks after the animals were moved to the enclosures. Samples from the control group in cages were taken for 4 weeks as well. The floors in the new enclosures were marked with 10 letters (A-J) and faecal samples were taken from each of these points every day for the first week and thereafter, every third day for the next three weeks.

In the enclosure of the initial female group, no distinct sampling points were available so faecal collection was carried out by chance based on a similar spatial distribution. All samples were collected in the morning hours before cleaning, placed in labelled plastic bags and frozen at -20° C until analysis.

2.4. Hormone Extraction

The hormone analysis was conducted at the Institute of Biochemistry at the University of Veterinary Medicine, Vienna. A group-specific enzyme immunoassay (EIA) for determination of 11.17-dioxandrostane (11.17-DOA) in hares (validated by Teskey-Gerstl et al., 2000) and for 17-OH-androstanes (both established by Palme and Möstl, 1997) was used. 0.5 g of faeces from each sampling point was diluted in 0.5ml distilled water and 4.0ml methanol 80%. After vortexing for 30 minutes and centrifugation for 15 minutes with 4000 rounds per minute, the tubes were frozen again at -20 °C until the assay was performed. After dilution of the supernatant with assay buffer (1:10) the samples were analysed.
2.5. Video Observation

Two webcams (Logitech C210, made in China, 640x480 dpi) were installed on the upper frame of the enclosures at a height of 2.4 meters. Due to limited lens coverage the entire enclosure could not be observed. Therefore the area with the most activity, as determined in a preliminary study, was chosen.

The video observation was started after several days of acclimatization in the new enclosures. Each group was recorded on three days for exactly three hours (9 hours in total) at the same time from the same camera positions. Video recording and editing was conducted with the open source software “virtualdub v1.9.11” (www.virtualdub.org). The three-hour video sessions were subsequently divided into 30-minute video sequences. All observations took place from March to May 2011.

2.6. Video Analysing Methods

Two different behaviour analysing methods were compared in order to select the most suitable and reliable method for the European hare. All behaviours were analysed in respect to the frequency of occurrence.

2.6.1. Continuous Sampling

All 30 minute video sequences were continuously watched and all behaviours recorded. During high activity periods the sequences were stopped every few seconds if necessary to record the individual behaviours of all participating hares. The number of defined behaviours per animal was counted.

2.6.2. Scan-Sampling

The behaviour patterns were noted in five minute time-intervals on still frames. In order to get an overview of the situation the video was stopped already five seconds before the fifth minute and was watched in slow motion until minute five was reached.
2.8. Conditions and Testing Procedures

Behaviours were defined and grouped in categories (see table 1). Certain behaviour was seen as consistent enough if it occurred at least once in every video session of three hours. Further analysing conditions were defined as follows:

1) If behaviour stops for more than three seconds it is considered to be finished.
2) If the same behaviour starts again after three seconds pause it will be counted as a new one.
3) Every bout of sniffing is counted, because this behaviour is performed in high frequencies.
4) “Sniffing at each other” will be counted as one event if two hares are stretching their necks towards each other.

Inner- as well as inter-observer reliability is regarded to be a quality measure of a parameter (Knierim & Winckler, 2009). Both were conducted in this study, first by the author for inner observer reliability, then two additional observers were trained for all relevant visible behaviours with six short training-video sequences. Thereafter 12 final-videos of five-minute duration each were selected (three out of every group) to assess inter-observer reliability.

2.9. Statistical Analysis

All statistical tests were performed with R 2.14.1 (R Development Core Team, Vienna, Austria). To identify the best fitting statistical models for the given data set the Akaike-information-criterion (AICc) corrected for small sample sizes was used. Thereby the model with the lowest AIC weight is the one with the highest probability to fit best to the given data set. Models with similar weights tend to be similarly supported by the AIC. If several models seemed plausible, a multimodel inference, like model averaging (Burnham and Anderson 2002), was performed, where average estimates from different model were used.

Repeated observations of different groups, sexes and dates warrant a veridical estimation of hormone levels and behaviours of the hares, but the potential effects of these fixed and random factors on the dependent variable, hormones and behaviour, had to be tested. Initially a large set of variables was included in every model to ensure that no effect was neglected. After evaluation of AICc, p-values for “significance” and “Relative Variable Importance”
(RVI) of estimated effects, variables were eliminated to avoid over-fitting. In not normally distributed residues Box- Cox- and log- transformations were performed.

To compare sex-specific differences in cortisol concentrations within the cage housing system and enclosures, an individual linear mixed-effects model was used with “date” and “sex” as fixed factor and the interaction of individual specific (cage) or point specific (enclosure) trends over “date” as random factor, (lmer \(\log(Cort_{ind} + 1) \sim \text{date} \times \text{sex} + (\text{date} | \text{Ind. Point}), \text{REML} = F\)) for each housing system. There was no effect in the combined testing with “date” and “sex”, so the dependency of cortisol was tested only with “sex” as fixed factor, lmer (formula = \(\log(Cort_{ind} + 1) \sim \text{Sex} + (\text{date} | \text{Ind. Point}), \text{REML} = F\)). For the baseline in cages the dependency of fGCM levels from “date” and “group” were tested with “date” and “group” as fixed factor. Interactions between different factors are always considered as random factors, so group specific trends over different dates served as random factor. Therewith a linear mixed effects model was formed, (lmer (log (Cort_ind +1) \sim \text{date}\ast\text{group} + (\text{date} | \text{Ind. Point}), \text{REML}=\text{F})).

In comparison to that an intercept-only-model (Random intercept model), (lmer (log (Cort_ind+1) \sim 1 + (\text{date} | \text{Ind.}), \text{REML}=\text{F})), was tested, in which predictors like “date” and “group” are ignored, but the random factor (date| Ind.) is allowed to vary.

Since the intercept only model got more support by AICc, the estimate of intercepts was used to determine mean values of cortisol in cages for every group. Confidence intervals were calculated by adding and subtracting standard errors to intercept values.

Female groups were analysed separately as no collection points were given for the first female group. No relevant differences between “collection points” at different “dates” were identified, which was confirmed by a variance of zero or close to zero for the random effects.

It was hypothesized that the translocation to the enclosures will increase the fGCM in the hares (translocation-peak), but that this phase would be followed by a decline of fGCM to a similar or lower stress level when compared to the cages. The hypothesis for the increase of cortisol at day one was tested by comparing the predicted ‘first-day’ GCM level with its upper and lower confidence intervals in the enclosures with the baseline (intercept) of this group within the cages. If the intercept fitted into the confidence intervals of day one values in the enclosure, there was no significant difference in stress levels.
A linear model with ‘date’ and ‘group’ as fixed factor was used to calculate fGCM concentration within the enclosure, \( \text{lm (formula = log (Cort\_ind + 1) \sim date * as. factor (group))} \). By predicting ‘first day’ values (\( \text{predict (m, interval="confidence", level=.95, data. frame (date=1, group="4")} \)) and the fGCM level at the last test day, using same approach, a trend for the fGCM-concentration development over the months was obtained.

If intercept values of fGCM in cage housing fit into confidence interval of enclosure values on the last testing day, the stress levels in both housing systems was thought not to differ. The last test day seemed the most meaningful, because animals should have habituated over the four weeks period to the new environment. Since there was no effect of “date” on hormone level in female group one (\( p_{\text{date}}= 0.587 \)), the intercept served to determine the fGCM level in this group.

In case of obviously non-linear or exponential trends over time, as in the second male group, a generalized additive model (GAM), as a curve of smoothed and additive functions, was used to display the trend over the month.

When putting males in a group housing system an increase in testosterone-metabolite and hence an increase in cortisol level was expected as testosterone may trigger behaviours that increase the stress level in the hares. Therefore the dependency of fGCM concentrations on testosterone was tested in a simple linear model with cortisol as dependent and testosterone-metabolite and group as fixed factors, \( \text{lm(formula = Cort\_ind \sim as. factor (group) * Test.}) \).

Likewise the dependency of the testosterone-metabolite on “group” and “date” was investigated with a simple linear model with “testosterone” as dependent variable and “date” and “group” as fixed factors, \( \text{lm(formula = log (Test. + 1) \sim date * as. factor(group))} \).

Since “date” had no effect on testosterone, fGCM level were estimated by a simple linear model with group as fixed factor, \( \text{lm (formula = log (Test. + 1) \sim as. factor (group))} \).

The control group in cages was tested in parallel to monitor potential seasonal effects on cortisol and testosterone. Corticosterone metabolite levels of these control animals were tested with a linear mixed-effects model with “date” and “sex” as fixed factors and the interactions of “date” and “individual” as random factors, \( \text{(lme (log (Cort\_ind+1) \sim date* sex, random= ~ date| individual, method= “ML”))} \). No effects were detected on fGCM metabolites from “date” and “sex” or from the interaction of “date” and “individual”. So an intercept-only model was used to estimate the control animal’s fGCM. To evaluate their testosterone-
metabolite level in male control animals, a simple linear model was used with testosterone-metabolite concentrations as dependent and “date” and “individual” as independent variables, lm (formula = Test. ~ date * Ind.). No effects of “date” and “individual” on testosterone-metabolite level could be seen, so an ‘intercept-only model’ was used again to measure testosterone-metabolite level of the control group, lm (formula = log(Test.) ~ 1).

Inner and inter-observer reliability testing was performed using Spearman rank correlations. Video observation analysing methods, continuous and scan sampling were compared using the Pearson- Correlation. Regressions were calculated beforehand and the residues checked visually.

To investigate the distribution and effects on the frequency of behaviours the independent variables “sex”, “day-time”, “date” and “group size” were considered. A general linear model with the frequency of “exploration”, “comfort”, “social” or “locomotion”- behaviour as dependent variables from given effects was used with the additional influencing variable “number of participating hares” as offset, glm (log (count of behaviour +1) ~ (date + time)*sex + group size, offset= number of participating hares). The proportions performance in behaviour categories in male and female hares were calculated in Excel.
3. Results

3.1 The Effects of Sex and Housing System on Hormonal State

Within the cages the effect of sex on fGCM-concentration of the study group was significant (p<0.01) with a variable importance of 0.85. Female hares had lower fGCM-concentrations than the males (esp. (beta) =0.54). The first female group showed a mean fGCM level of 13.19 ng/g in mean [CI 7.55 ng/g; 23.04 ng/g], the second female group of 11.59 ng/g in mean [CI 7.55 ng/g; 15.63 ng/g]. Higher fGCM concentrations were found in the males with 20.31 ng/g in mean [CI 12.87 ng/g; 34.62 ng/g] with no significant differences in both groups.

Faecal GCM-levels in cages served as a baseline to compare the stress situation within the enclosures on the first day after release. Faecal GCM-levels increased slightly but significantly in the first female group from 13.19ng/g in mean [CI 7.55 ng/g; 23.04 ng/g] in cages to 16.19 ng/g in mean [CI 19.76 ng/g; 13.26 ng/g] in the enclosures. In the second female group the faecal Glucocorticoid Metabolite level rose noticeably from 11.59 ng/g in mean [CI 7.55 ng/g; 15.63 ng/g] in the cages to 27.93 ng/g in mean [CI 22.88 ng/g; 34.09 ng/g] on the first day in the enclosure. This trend was also observed in both male groups, with an increase in fGCM from 20.31 ng/g in mean [CI 12.87 ng/g; 34.62 ng/g] in the cages to 57.65 ng/g in mean [CI 47.05ng/g; 70.62ng/g] and 59.94 ng/g in mean [CI 40.52 ng/g; 88.66 ng/g] respectively on the first day in the enclosures. Thus an increase of fGCM could be seen in every group.

After the habituation-peak the level of fGCM-concentrations in all 4 groups during the test month showed a heterogeneous distribution. The first female group remained constant to “day one” levels in the enclosures over the entire month with a slope close to zero (n= 0.0023). No relevant effect of “date” could be found in this group. Hence fGCM level in this group was slightly but significantly higher within the enclosure (16.19 ng /g) when compared to their single housing system (13.19 ng/g). Faecal GCM declined exponentially in the second female group, but not significantly under cage level (n = - 0.035). The first male group dropped exponentially and significantly under cage level (n= -0.041). Mean values for the last two groups at the end of the test month in the enclosures were 10.96 ng/g in mean [CI 8.27 ng/g; 14.52 ng/g] (11.59 ng/g within the cages) and 12.18 ng/g in mean [CI 9.20 ng/g; 16.12 ng/g] (20.31 ng/g within the cages) respectively.
A considerable discrepancy was noticed in the second male group. Neither a linear nor an exponential trend could be seen in this group. Faecal GCM levels dropped sharply after the habituation-peak of day one to around cage level. A period of stability with negligible fluctuations around this cage level followed for the next 3 weeks. Suddenly the fGCM levels rose continuously and significantly for the rest of the month.

The control group showed an fGCM level of 23.57 ng/g with no difference between sexes.

**Figure 1: Faecal Glucocorticoide Metabolite Development (fGCM) during test month for test-groups, red lines are female groups, green lines are male groups**

![Development of hormone level](image)

A strong positive correlation was observed between cortisol and testosterone-metabolites with $p = 6.5 \times 10^{-13}$ and a relative variable importance of RVI = 0.95. The testosterone-metabolite increased slightly, but significantly, from a concentration of 11.22 ng/g in mean [9.94 ng/g; 12.5 ng/g] within the cages to 14.41 ng/g [13.4 ng/g; 15.4 ng/g] in the enclosures with a noteworthy stability over time as seen in coefficients with no effect of “date” on testosterone-metabolite values ($p_{date}=0.221$). The effect factor “group” was highly significant with $p_{group} = 2 \times 10^{-16}$. The second male group demonstrated a significantly higher testosterone-metabolite level when compared to the first male group in the enclosures ($p = 2 \times e^{-16}$). The control animals in cages showed a testosterone- metabolite level of 10.12 ng/g in mean [CI 8.99 ng/g; 11.26 ng/g], which is not a significant difference to the testosterone level.
of the male study animals in cages, but a significant difference to the values of the study groups in the enclosures.

3.2. Definition of Behaviour

The process of defining behavioural parameters started with elaborating an ethogram of the hares in all video clips. Some behaviour was rarely seen, e.g. “boxing” occurred only two times in total in all videos and did not seem consistent enough to be included in the protocol. In a second step the revised list of observed behaviours included all behaviour patterns visible for an observer excluding boxing, urinating, mounting and feeding. Feeding, even though an interesting behaviour, could not be monitored because the food hoppers were placed outside of video coverage on the wall. The final descriptions on the observed behaviour are summarized in Table 1.

**Table 1: Definition and Categories of Behaviour in the European Hare**

<table>
<thead>
<tr>
<th>Comfort Behaviour</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grooming</td>
<td>Hind legs scratching the ear, licking and arranging the fur with the tongue and teeth, forelimbs groom head and ears.</td>
</tr>
<tr>
<td>Stretching</td>
<td>Forelimbs pull the body forward to an elongated posture the hind limbs stay in a fixed position.</td>
</tr>
<tr>
<td>Resting</td>
<td>Sitting on the floor and adducting all legs near the body, the back is rounded and the ears are flattened on the body.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Exploration Behaviour</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sniffing</td>
<td>The hare puts the nose to the floor or to an enclosure item to smell/perceive an odour. While sniffing forward movement is possible.</td>
</tr>
<tr>
<td>Stand up on hind legs</td>
<td>The hare balances its body weight on the hind legs and takes the forelimbs off the floor in a look-out position. Contact of the forelimbs with the enclosure wall is possible, if they trying to look across the sidewall.</td>
</tr>
<tr>
<td>Listening</td>
<td>Ears are erected; at least 45 degrees; head and upper part of the body are elevated for a better overview. Forelimbs are extended to support the risen body.</td>
</tr>
</tbody>
</table>

**Socio Positive Behaviour**
Sniffing each other  Extending the nose from one or both hares to an group member in a distance of maximum 50 cm.

<table>
<thead>
<tr>
<th><strong>Agonistic Behaviour</strong></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Chasing</td>
<td>The hare runs fast behind a group member and chases it for more than four hops. No contact occurs.</td>
</tr>
<tr>
<td>Fleeing</td>
<td>Fleeing a chasing group mate or to shying away from an approaching hare.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th><strong>Locomotion</strong></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Hopping</td>
<td>Going from one place of the enclosure to another with more than two hops.</td>
</tr>
</tbody>
</table>

3.3. *Inner - Observer - Reliability for Behavioural Parameters*

From the first definition of parameters to the last revised edition the mean correlation values of counts of certain behaviours from the protocol improved from of $r_s = 0.76$ to $r_s = 0.93$ (Table 2).  

**Table 2: Inner-Observer-Reliability (IOR) - Test Results for the First Defined Behaviour Description and for the Last Revised Edition.**

<table>
<thead>
<tr>
<th>Behaviour</th>
<th>First Version</th>
<th>Revised Version</th>
</tr>
</thead>
<tbody>
<tr>
<td>grooming</td>
<td>0.92</td>
<td>0.97</td>
</tr>
<tr>
<td>stretching</td>
<td>0.88</td>
<td>0.97</td>
</tr>
<tr>
<td>sniffing</td>
<td>0.63</td>
<td>0.96</td>
</tr>
<tr>
<td>stand on hind legs</td>
<td>0.23</td>
<td>0.94</td>
</tr>
<tr>
<td>sniffing each other</td>
<td>0.82</td>
<td>0.89</td>
</tr>
<tr>
<td>chasing</td>
<td>0.91</td>
<td>1</td>
</tr>
<tr>
<td>fleeing</td>
<td>0.90</td>
<td>0.98</td>
</tr>
<tr>
<td>hopping</td>
<td>not analysed</td>
<td>0.84</td>
</tr>
<tr>
<td>resting</td>
<td>not analysed</td>
<td>0.85</td>
</tr>
<tr>
<td>listening</td>
<td>not analysed</td>
<td>0.97</td>
</tr>
<tr>
<td>mean</td>
<td>0.76</td>
<td>0.93</td>
</tr>
</tbody>
</table>
3.4. Inter-Observer-Reliability (IOR)

Mean values of IOR for training and test videos are shown in Table 3 and Table 4. In summary an increase in correlation of test results could be achieved with the inter-observer training from \( r_s = 0.93 \) to \( r_s = 0.94 \) in the final testing.

**Table 3: IOR-Test Results for Training Videos using Spearman Rank-Correlation Coefficients**

<table>
<thead>
<tr>
<th>Behaviour</th>
<th>Observer1/Observer2</th>
<th>Observer1/Observer3</th>
<th>Observer2/Observer3</th>
<th>mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>grooming</td>
<td>0.90</td>
<td>0.98</td>
<td>0.88</td>
<td>0.92</td>
</tr>
<tr>
<td>stretching</td>
<td>0.71</td>
<td>1.00</td>
<td>0.71</td>
<td>0.80</td>
</tr>
<tr>
<td>sniffing</td>
<td>0.94</td>
<td>1.00</td>
<td>0.94</td>
<td>0.96</td>
</tr>
<tr>
<td>Stand on hind legs</td>
<td>1.00</td>
<td>0.98</td>
<td>0.98</td>
<td>0.99</td>
</tr>
<tr>
<td>Sniffing each other</td>
<td>0.71</td>
<td>0.71</td>
<td>1.00</td>
<td>0.80</td>
</tr>
<tr>
<td>chasing</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>fleeing</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>hopping</td>
<td>0.99</td>
<td>0.95</td>
<td>0.94</td>
<td>0.96</td>
</tr>
<tr>
<td>resting</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td></td>
</tr>
<tr>
<td>listening</td>
<td>1.00</td>
<td>0.99</td>
<td>0.99</td>
<td>0.99</td>
</tr>
<tr>
<td><strong>mean</strong></td>
<td><strong>0.9</strong></td>
<td><strong>0.95</strong></td>
<td><strong>0.93</strong></td>
<td><strong>0.93</strong></td>
</tr>
</tbody>
</table>
TABLE 4: IOR-TEST RESULTS FOR FINAL TEST VIDEOS USING SPEARMAN RANG CORRELATION COEFFICIENTS

<table>
<thead>
<tr>
<th>Behaviour</th>
<th>Observer1/Observer2</th>
<th>Observer1/Observer3</th>
<th>Observer2/Observer3</th>
<th>mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>grooming</td>
<td>0.88</td>
<td>1.00</td>
<td>0.88</td>
<td>0.92</td>
</tr>
<tr>
<td>stretching</td>
<td>0.99</td>
<td>0.99</td>
<td>1.00</td>
<td>0.99</td>
</tr>
<tr>
<td>sniffing</td>
<td>0.97</td>
<td>0.98</td>
<td>0.93</td>
<td>0.96</td>
</tr>
<tr>
<td>Stand on hind legs</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>Sniffing each other</td>
<td>1.00</td>
<td>0.95</td>
<td>0.96</td>
<td>0.97</td>
</tr>
<tr>
<td>chasing</td>
<td>0.90</td>
<td>1.00</td>
<td>0.90</td>
<td>0.93</td>
</tr>
<tr>
<td>fleeing</td>
<td>1.00</td>
<td>0.99</td>
<td>0.99</td>
<td>0.99</td>
</tr>
<tr>
<td>hopping</td>
<td>0.86</td>
<td>0.86</td>
<td>0.93</td>
<td>0.89</td>
</tr>
<tr>
<td>resting</td>
<td>0.85</td>
<td>0.85</td>
<td>1.00</td>
<td>0.90</td>
</tr>
<tr>
<td>listening</td>
<td>0.81</td>
<td>0.84</td>
<td>0.78</td>
<td>0.81</td>
</tr>
<tr>
<td>mean</td>
<td>0.93</td>
<td>0.95</td>
<td>0.94</td>
<td>0.94</td>
</tr>
</tbody>
</table>

3.5. Correlation of Sample Scans and Continuous Observation

There were positive correlations of test results in all defined behaviour categories achieved by continuous video analysis and scan sampling. The lowest relation was found in the parameters ‘socio-positive behaviour’ (r= 0.15; p= 0.383 for males and r= 0.01; p= 0.95 for females) and ‘agonistic behaviour’ (r= 0.27; p= 0.11 in males, female hares never showed agonistic behaviour). Moderate correlation was found for ‘comfort behaviour’ (r= 0.55; p= 0.001 for males and r= 0.62; p= 0.000 for females). ‘exploration behaviour’ correlated low for the males but very good for the females (r= 0.36; p= 0.03 and r= 0.73; p= 0.00, respectively) similar to ‘locomotory activity’, correlating low for the males but good for the females (r= 0.14; p= 0.41 and r= 0.67; p= 0.000, respectively).
3.6. Distribution of Behaviour Patterns

The effect of “sex”, “time” (earlier or later afternoon hours), “date” (progress over the month) and “group size” (animals per group) on behaviour was tested by evaluating p-values and relative variable importance. The “date:sex” interaction (p= 0.01; RVI= 0.84) is relevant for exploratory behaviour. This behaviour increased slightly in females during the study period. For locomotory activity the variables “time” (p= 0.02; RIV= 0.89) and “date:sex” interactions (p= 0.003; RIV= 0.96) were important. This behaviour occurred more frequently in the later afternoon hours in both sexes. The effect of “date” in interaction with “sex” showed an increase in locomotory behaviour for female hares during the three observation days.

A substantial effect on comfort behaviour can be seen in the variable “sex” (p= 0.00; RIV = 1.00), “sex:time” interaction (p= 0.001; RIV= 0.99) and “group size” (p= 0.01; RIV= 0.95). Female hares performed more comfort behaviour than males. This behaviour increased during the afternoon hours in female groups. The bigger the group size the less comfort behaviour was seen for male hares.

Major effects on agonistic interactions can be seen in the variables “sex” (p= 5.82 *10^-5; RVI= 1.00), “time” (p_{time}= 0.019) and “group size” (p= 0.040). This behaviour was never seen in female groups. An increasing trend in males was recognized during afternoon hours and with group size. The degree of socio positive behaviours is influenced by “sex” (p= 9.49*10^{-5}; RVI= 1.00) and “sex:time” interaction (p= 4.22*10^{-5}; RVI= 1.00). The inter-individual approach presented as “sniffing each other” was seen more often between female hares and increased during the afternoon hours.

3.7. Proportions of Behaviour Categories

Taking both sexes together, the most frequently observed behaviour categories were exploration and locomotion with 50% and 33.8% respectively. Comfort behaviour followed with 6.3%. Agonistic and socio positive behaviour occurred rarely only with 6.1% and 2.6% respectively (Figure 2).
Figure 2: Percentages of behaviour categories (for female groups in red columns for male groups in blue columns)
4. Discussion

4.1. Hormone Investigations

The stress level of wild animals, particularly of prey species like hares, cannot be easily evaluated by direct observation. The measurement of faecal glucocorticoid metabolite (fGCM) concentrations, a non-invasive method that is at present well-established and widely used for various domestic and non-domestic species proved to be a reliable indirect indicator of stress (Touma and Palme, 2005; Monclus et al. 2006; Teskey-Gerstl et al., 2000). Certainly, individual differences in fGCM concentrations due to a variety of factors such as sex, age and social rank, individual resilience of the animal and body condition can occur (Naguib, 2006). Group and sex specific differences in fGCM - concentrations and behaviour may be influenced by individual variations in responding to new challenges in the environment (Naguib, 2006). Sex specific differences in glucocorticoid excretion are known for various species (Touma and Palme, 2005). A previous study examining the fGCM levels in urine and faeces of the European hare (Lepus europaeus) showed that female hares tended to have a higher level (Teskey-Gerstl et al., 2000). Other studies evaluating the fGCM level in snowshoe hares (Sheriff et al., 2009) and wild rabbits (Monclus et al., 2006) could not detect a difference between the sexes. In this study female hares in cages showed only half the fGCM concentration than the males (13.19 ng/g and 11.59 ng/g for the females; 20.31 ng/g for the males), however no significant sex differences (p= 0.375) could be seen in the control groups in cages (joint fGCM level of 23.57 ng/g).

Interestingly the second male group showed a significantly higher testosterone-metabolite level compared to the first male group. This may explain the sudden increase in fGCM levels in this group at the end of the month, even though there was no significant effect of ‘date’ on testosterone levels.

A clear effect of translocation could be seen in all study animals. After the first day in the enclosures both sexes showed an increase in fGCM, indicating the expected increased stress level due to the translocation, the new environment and intra-specific contact.

In various studies translocation (capture, transport and release in unknown habitats) is clearly shown to be a stress factor in many animal species including hares (Paci et al, 2004, Vick et al., 2012, Reimers et al., 2007, Buijs et al., 2011). A study of cows by Higashiyama et al. (2007), showed an approximately 3.4 fold increase in cortisol levels over the first week when cows (Bos primigenius taurus) were moved from pasture to indoor tethering. Persian onager
Equus hemionus onager showed a two times higher fGCM level when moved from spacious pasture to small yards combined with human presence (Vick et al., 2012). In both studies the levels rapidly declined later on. In contrast, in a study by Chelini et al, 2011, fGCM – concentrations remained unaffected by housing conditions and social rank for Syrian hamsters (Mesocricetus auratus). Also forming pairs of these solitary living animals did not influence fGCM concentrations.

Short term increases in fGCM levels can also be adaptive (Moberg, 2000; Bassett and Buchanan-Smith, 2007) and therefore should not at all times be regarded as harmful (Bassett and Buchanan-Smith, 2007). Agonistic behaviour, but also increased reproductive activity or exploration can cause an elevation of glucocorticoid metabolites (Breuner, 1998; Sandi et al., 1996).

Although faecal sampling occurred around the same daytime, not all droppings were collected for analysis. Some authors found high correlations between faecal GCM-concentrations in a representative samples and the absolute amounts of excreted glucocorticoid metabolites (Lepschy et al, 2010). Others advise to collect all faeces for a more accurate measurement using the absolute amounts of excreted metabolites (Hau et al, 2011).

It is mentioned by Cavigelli et al, 2005 that Corticosterone from the blood enters the small intestines, after metabolised by the liver, independently from the amount of feeding, but concentrations of fGCM vary according to the quantity and motility of gut contents. In the new environment of the enclosure animals may not find the feeding lots immediately and therefore excrete fewer amounts of faeces with higher concentrations of fGCM.

Potential diurnal changes in faecal GCM concentrations were investigated by Sheriff et al., 2009, in snowshoe hares (Lepus americanus). In their study the absolute amount of injected “radioactive cortisol” showed a clear diurnal rhythm in its excretion, since these animals void the most faeces during night hours. However the amount of radioactivity per gram of faces did not changed over the day. This fact makes sure that there will be no effect of time of the day on the fGCM concentrations in the droppings, because collection was done only once a day in the morning and samples collected are consequently passed on different time of day.

Not all groups were tested in the same month, so variations of glucocorticoid level over the year also need to be considered. But evidently significant seasonal changes of glucocorticoid excretion could neither be found in mountain hares in the field (Rehnus et al., 2010) nor in European hares in captivity (Janicki et al, 2006).
4.2. Behavioural Investigations

During the process of developing a precise behavioural description, using the inner-observer correlation, reliability increased. Major progress was made by narrowing a term down in time and space, for example determining behaviour as finished following a three second interruption, giving “sniffing each other” a minimal distance of 50 cm or minimizing ‘chasing’ to at least four hops.

As pointed out previously, observers should get used to the identification and conditions of every behaviour pattern during training (Martin and Bateson, 1993). Inter-observer reliability results improved in this study from the training-video sequences to the final-videos. In sequences with a lower activity and presence of hares were chosen for that initial training. In contrast, video clips with high activity and mostly all group members present were used for the final testing. The inter observer reliability for all behaviours was high to very high (0.7-1.0) according to Martin and Bateson, 1993.

4.3. Performance of Behaviour

In the open and spacious housing system hares demonstrated a complex spectrum of behavioural expressions. Even though it is not possible to give a comparison to cage housing, we assume that most of the behaviours shown within the enclosures were not practicable in the cages.

The comfort behaviour is a good indicator for animal welfare considered by the author. Since the group size affects comfort behaviour negatively, the number of animals in groups seems to be of high relevance.

Agonistic behaviours were rare, even though male and female groups were kept next to each other. This might indicate that intra-specific conflicts in the breeding season may not occur if sexes are separated and genders do not see each other. Since agonistic behaviours also seem to be negatively influenced by group size, small group sizes in male groups are recommended. Physical contact between animals occurred only in rare instances. In some cases agonistic behaviour (represented by chasing and fleeing) was even difficult to distinguish from play and voluntary following of a mate.

Various influence factors such as availability of different structure materials, hiding places and possibilities for escaping from group mates may play a role. Environmental enrichment could mitigate stress of the animals by increasing the possibilities to express species-specific behaviour (Baumans, 2005). Since hares prefer open, but scattered landscapes with hedges
and shrubs as hiding possibility (Chapman and Flux, 1990; Corbet and Harris, 1991) these fitments should be considered when designing enclosures. In Ferretti et al. (2010) hares showed an average daily movement of only 35 meters (±11.9m) for resident hares and 53 ± 9.2 m for relocated hares. Other scientists may evaluate bigger ranges of activity during the day (Rühe and Hohmann, 2004), but apparently hares hence do not require huge dimensions in their housing system. More important is the arrangement of the space provided rather than its dimension (Baumans, 2005).

The absence of human presence is an important factor for habitat selection as also described by Marco Ferretti et al, 2010. It is also demonstrated with other wild animals, which show higher glucocorticoid secretion due to the influence of human disturbance (Dehnhard et al. 2001; Arlettaz et al., 2007). Cage housing may offer more security from potential predators and consequently reduce stress. However a big advantage for the animals in the enclosures is the possibility to get out of human proximity and to elope when keepers perform the daily cleaning, which is not possible during cage cleaning. Morgan and Tromborg (2007), who analysed sources of stress in captivity, described that the lack of possibilities to escape is a severe stressor for animals in captivity. Also the predictability of an approaching human is assumed to reduce stress for captive animals (Bassett and Buchanan-Smith, 2007).

The effects of the new housing system on hares behaviour are not predictable and constant for all groups, but influencing factors like for the group size, age-composition and even animal’s genetics can be optimized.

4.4. Analysing Methods

The author considers continuous video watching as the most exact way to reflect a given video content and recommends, evidentiary by the results, to take this method to display the real behaviour of this species. Only for the explorative and locomotory behaviours, which would yet represent at least 90% of females’ behaviour, correlations were good enough to use scan sampling. Another more time saving approach would be to shorten the time interval for the scan sampling to lower than five minutes. Scan sampling requires much less time to get an overview of behaviour, but especially for the males short lasting behaviours, like “chasing” and “fleeing” may not be seen at any measuring point. This would distort the actually picture of hares behaviour in this housing system.
5. Conclusion

Overall this study shows that group housing for the normally solitary living hares has potential as an animal welfare friendly system. Three out of four groups in the enclosures dropped back to cage levels of fGCM after habituation, despite fluctuations occurring at the end of the test month. The forth group showed fGCM level comparable to cage concentrations in the enclosures for the whole month. The new housing system provides an open, structured and spacious environment where locomotory activity and augmented normal behaviour can be and are performed.
Acknowledgement

I appreciated the help of Peter Steiger and Michaela Salaba for collecting samples and the assistance of Elke Leitner in biochemical analysis.
References


Zusammenfassung

Im Rahmen der Arbeit wurden die Effekte zweier Haltungssysteme auf das Wohlbefinden des Europäischen Feldhasen (*Lepus europaeus*) am Forschungsinstitut für Wildtierkunde und Ökologie untersucht. Die zu vergleichenden Haltungssysteme sind zum einen konventionelle Stoffwechsel-Käfige, in denen die Tiere individuell untergebracht sind, und neu entwickelte ausgestaltete Gehege zur Gruppenhaltung.


Vier Versuchsgruppen (zwei männlich und zwei weiblich) wurden für diesen Versuch zusammengestellt. Diese setzten sich aus 20 Tieren zusammen, die zur Versuchszeit in keinen anderen Studien eingebunden, nicht trächtig und gesund waren. Männliche Gruppen (je vier Tiere) wurden kleiner als weibliche Gruppen (je sechs Tiere) gewählt, da bei den männlichen Tieren mögliche Revierkämpfe zwischen den Tieren nicht ausgeschlossen werden konnten.


auffällend in allen Gruppen. Das Testosteron der männlichen Tiere erhöhte sich signifikant in den Gehegen, blieb dort jedoch stabil über die Zeit und korreliert signifikant mit den fGCM-Konzentrationen.


Das Verhaltensrepertoire der Hasen gestaltet sich sehr vielseitig. Verhalten aus den Kategorien Lokomotion, Erkundung, Komfort, Sozio-positives und agonistisches Verhalten wurde gezeigt. Die häufigsten Verhaltensmuster waren Erkundung mit etwa 50 % und Lokomotion mit 38% aller Verhaltensweisen. Diese werden gefolgt vom sehr viel selteneren Komfort- (6,3%), agonistischem (6,1%) und sozio-positivem (2,6%) Verhalten. Das antagonistische Verhalten wird durch „Verfolgen“ und „Flüchten“ definiert und ausschließlich von männlichen Tieren gezeigt.

Weitere Einflussfaktoren auf das Verhalten wie Gruppengrösse, Geschlecht, Uhrzeit und Versuchszeitraum, sowie dessen Interaktionen wurden untersucht.

Die Studie leistet einen wichtigen Beitrag zur Evaluierung und Optimierung von potentiellen Haltungssystemen beim europäischen Feldhasen.