Physiology & Behavior 104 (2011) 469–473

Contents lists available at ScienceDirect

Physiology & Behavior

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

Glucocorticoid metabolites in rabbit faeces—Influence of environmental enrichment and cage size

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ARTICLE INFO

Article history:
Received 30 July 2010
Received in revised form 13 April 2011
Accepted 6 May 2011

Keywords:
Faeces
Corticosterone
Enrichment
Cage size
Rabbit
Transport

ABSTRACT

The concentration of glucocorticoid metabolites (GCM) in rabbit faeces has been suggested as a non-invasive indicator of stress. In the present study, GCM concentrations were measured in faeces of fattening rabbits kept in groups of eight, at seven different stocking densities (between 5 and 20 animals/m^2), with or without environmental enrichment (a wooden structure used mainly for gnawing and resting). Transport (30 min) was used as an acute novel stressor to assess the glucocorticoid response to stress under the different housing conditions. GCM concentrations were elevated post-transport ($P<0.001$). Whilst cage size had no influence on GCM, enrichment reduced GCM concentrations before as well as after transport ($P=0.007$ in both cases). Effects of cage size and enrichment on growth characteristics were negligible, whilst enrichment decreased cage manipulation and social contact. The results indicate that even short transport durations may be stressful for rabbits, and that enrichment may decrease housing stress. They suggest that measuring baseline GCM concentrations in faeces is a useful tool to evaluate chronic stress in rabbits, whilst measuring the response to a novel stressor did not provide additional insight.

1. Introduction

Chronic stress has been defined as a series of acute stressors whose accumulated biological costs force animals into a pathological or pre-pathological state [1]. Chronic stress is of particular interest to the study of animal welfare because it can lead to depression of the immune and reproductive systems, as well as to alterations of brain structures that result in impairment of functions and mood disorders [1–3]. There is some controversy regarding the effect of chronic stress on glucocorticoid (GC) levels. Some have used elevated GC concentrations to define chronic stress [4]. Others claim that baseline concentrations are not elevated during chronic stress, but that the sensitivity of the hypothalamic-pituitary-adrenal (HPA)-axis is altered. This would in turn lead to either an amplification [3,5] or suppression [6–8] of the GC response to stressors different from the one that caused the chronic stress. Also, there is some debate on how this response should be defined. Some authors simply use concentrations after the novel stressor [9–11], whilst others used the difference between post-stressor and baseline concentrations [8,12] or the proportion of the increase from the baseline sample [13].

Although blood samples are most commonly used for the analysis of GC concentrations, blood sampling itself can induce increased GC concentrations [14,15]. Furthermore, circadian variations [16], pulsatile secretion and short term stressors [2] influence blood GC concentrations. These effects are thought to be attenuated when glucocorticoid metabolites (GCM) are measured in faeces samples because faeces can be collected without disturbing the animal, and because faecal GCM reflect the accumulation of glucocorticoids over several hours [2,15,17]. Small changes in baseline concentrations resulting from chronic stress that are not detectable by blood analysis might therefore be detected by quantifying faecal GCM [5]. Although individual variation in faecal GCM is usually large, using each animal as its own control can reduce this influence [18]. Increased baseline faecal GCM concentrations have been reported for rabbits when exposed to predator odour [19] and when predator pressure increased [20]. Examples in other species include increased CGM metabolites in zoo-kept rhinoceroses and leopards in response to increased exposure to the public [21,22].

Space allowance can influence GC levels in the blood [11,13,23], although this effect may be species specific. Increasing floor space allowance reduced faecal GCM concentrations in margay (*Leopardus wiedii*), but not in tigrinas (*Leopardus tigrinus*) [24], mink [25] or chickens [26,27]. Fattening rabbits are usually housed at a stocking density of 14–20 animals/m^2, in groups of 2 to 6 individuals [28]. A recent study (Buijs et al., unpublished data) showed that nine-week old rabbits avoided each other's proximity even when stocked at 5 animals/m^2, indicating that they would prefer to have more space. Small cages and high stocking densities are also reported to limit

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doi:10.1016/j.physbeh.2011.05.008

rabbits' locomotory, social and resting behaviour as well as their behavioural variability, and increase fearfulness, aggression, and redirected grooming and cage manipulation [28,29]. Thus, effects on GC may be expected as well, but little is known about this to date. Higher baseline plasma corticosterone concentrations have been reported for rabbits housed in groups of 5 than for those housed in smaller groups [30], but since group size was confounded with stocking density it is unclear which of these two factors caused the corticosterone increase. However, no difference was observed between rabbits housed individually or in groups of eight individuals when stocked at equal density [31], supporting density as the key factor.

Environmental enrichment is thought to alleviate stress in domestic animals, by making the environment more controllable or stimulating, and by increasing the possibilities to express species-specific behaviour [32], thus influencing GC levels [33–35]. However, some studies report that enrichment decreases baseline GC levels [10,36], whilst others report no effect on baseline levels, but a decreased [33] or increased [8] response to a novel stressor. Some of the differences between studies may be explained by the different species, enrichment strategies and experimental protocols used. But until more is known about the effects of chronic stress on the GC mechanism, it remains important to measure baseline concentrations as well as response to novel stressors. Studies measuring the effect of enrichment on faecal GCM are usually limited to baseline values, and have led to contradictory results. For chickens, enrichment led to increased baseline GCM concentrations [37], but for tigrinas [24] and mink [25] enrichment decreased baseline concentrations. Even within species differences may occur, as some maned wolves were reported to respond to enrichment with an increase in baseline faecal GCM, whilst in other individuals GCM decreased in response to the same enrichment [38].

In the only study on the effects of enrichment on GC in rabbits, no differences in blood corticosterone concentrations were found for rabbits housed individually in barren cages and those in enriched group cages [31]. However, the enrichment did not include gnawing materials, the most common type of enrichment for rabbits. Wooden sticks have been reported to reduce bar-biting, aggressiveness, alertness and self grooming, to increase allogrooming, social contact and hopping, and to improve some production characteristics, although these results are not consistent between different studies [39]. The housing system (individual vs. group-housed) and type of material influence gnawing material usage [40,41] and may be at least partially responsible for the inconsistencies between studies [39]. There is therefore a need for further studies on the effect of enrichment on GC in rabbits.

The aim of this experiment was to evaluate the effect of increased cage size and environmental enrichment on rabbits' baseline GCM concentrations as well as their GCM response to a novel stressor. Transport was chosen as stressor because of its practical relevance, and because it increases blood GC concentrations in rabbits [42] and faecal GCM in other species [21,43]. To our knowledge, the effect of providing gnawing material on rabbit GC levels has not been studied before. The expectation was that smaller cages would lead to increased stress levels, expressed by a higher GCM baseline or an altered GCM response to transport. In contrast, enrichment was expected to alleviate some chronic stress and thus decrease baseline GCM concentrations and the response to transport. In addition the effect of cage size and enrichment on behaviour, average daily gain and feed conversion were scored.

2. Methods

2.1. Animals and housing

Crossbred meat type rabbits (n = 672, Dams: New Zealand White × Californian, Sires: New Zealand White and Large Butterfly, the last breed was used to obtain progeny with different fur colours to facilitate handling and behavioural observations) were bred at the test facility. Kits were handled twice in the first week post-partum: picked up from the nest, stroked, and kept in a container with their siblings for about 2 min. This was done to decrease fear of humans [44] and thus increase ease of handling during the experimental period, during which the animals had to be handled repeatedly to assess production characteristics. At 30 days of age the rabbits were weaned, tattooed in the ear for individual recognition and distributed over the experimental open-top wire cages in groups of 4 males and 4 females. No siblings were present in any one cage and the distribution of the animals over the treatments was balanced for genotype. Cages were 100 cm long and 160, 107, 80, 64, 53, 46 or 40 cm wide, resulting in stocking densities of 5; 7.5; 10; 12.5; 15; 17.5 and 20 animals/m^2, respectively. With the exception of the cages of 46 and 40 cm wide, half of the cages were enriched with a ⊔ shaped wooden structure (40 × 20 × 20 cm, l × w × h) that could be used as a shelter, gnawing substrate, and as a way to avoid contact with the wire floor. Physical space limitations meant that no enrichment was offered in the smallest two cage sizes. The study consisted of 3 experimental replicates, and in total 12 unenriched cages were set up for each of the two smallest pen sizes, and 6 enriched and 6 unenriched cages for each of the five largest cage sizes. Dead animals were replaced during the first two weeks of the rearing phase. No later replacements were made to avoid disruption of the social structure within the groups which may alter GC levels. Ambient temperature was kept at 20 °C throughout the fattening period by means of central heating and air conditioning. A light schedule of 8 h light, 12 h dark and 2 × 2 h of twilight (decreased light intensity) was used (twilight: 6 am–8 am, light 8 am–4 pm, twilight 4 pm–6 pm, dark 6 pm–6 am). Water and food (a commercial rabbit fattening diet) were available ad libitum from 2 nipples and 4 feeders per pen, respectively.

2.2. Transport and faecal sample collection

At 75 days of age (i.e., around commercial slaughter age) individual animals were carefully picked up from their cages, crated together with their cage mates, transported in a van for 30 min, and then returned to their cages. Faeces were collected by placing fine wire netting boxes underneath the cages. As such, each sample contained faeces from all animals in the pen. Urine could pass through the netting, thus separating it from the faeces. Collection took place from 18 to 8 h before transport, and from 6 to 16 h after transport (thus, at the same time of day). This schedule was chosen to assure that each individual in the pen would excrete its peak GC sample within the collection period. Faecal GCM are reported to peak around 12 h after an acute stressor, but some individuals deviate from this mean [19]. After collection, samples were frozen directly and stored at −20 °C until analysis.

2.3. Glucocorticoid analysis

Faecal samples were analysed using a 5α-Pregnane-3β,11β,21-triol-20-one enzyme immunoassay (EIA). Since rabbits produce both corticosterone and cortisol [14,45,46], an antibody that picks up the metabolites of both hormones was used. This antibody had previously been proven suitable for quantifying GC metabolites in faeces of rabbits [19], mountain hare [47], and a number of rodents (mice: [15]; rats: [48]; ground squirrels: [49]). In brief, 0.5 g of homogenised faeces was suspended in 80% (v/v) methanol and shaken for 30 min. After 1:100 dilution with assay buffer, duplicates of 50 µl each were measured in the EIA as described in detail by [50]. Intra- and interassay variations were 10% and 11%, respectively.

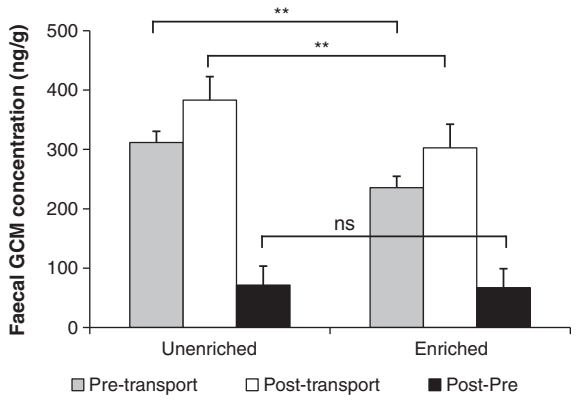

Fig. 1. GCM concentrations in faeces of rabbits housed without and with environmental enrichment (LSMEANS + SEM). Faeces were collected before and after a novel stressor: 30 min of transport.

2.4. Behaviour

Video recordings were made at six and nine weeks of age. Each cage was recorded three days per week, nine times per day. Behaviour was analysed by scan sampling the number of animals performing lying, stationary (sitting or standing), locomotor (hopping, running, frolicking and rearing), ingestive (eating and drinking), social (sniffing, licking, nibbling and grooming another rabbit), comfort (self-grooming and stretching), cage manipulation (excluding manipulations of the enrichment), and agonistic (aggressive and avoidance) behaviour. Occurrence was averaged per scan and subsequently averaged over the nine observations per day.

2.5. Growth characteristics

Average daily gain was measured on an individual level by weighing the animals at 30, 44, 58, 63 and 70 days of age. Feed conversion was determined on the same days, but was measured at the pen level because feed intake could not be determined individually since all animals in one cage could eat from all four feeders.

2.6. Statistical analysis

All statistics were performed in SAS 9.1.3. A paired t-test was used to compare pre-and post-transport GCM concentrations. The response to transport was calculated in two ways using each cage as it own control: by subtracting pre-transport GCM concentrations from post-transport ones, and by dividing post-transport concentrations by pre-transport ones. To study the effects of housing, GCM concentrations were analysed on cage level, treating duplicates as repeated measures, in a mixed model including cage size, enrichment and their interaction as fixed factors and experimental round as a random factor. As only unenriched cages were available for the smallest two cage sizes, analyses of the enrichment effect and the enrichment × cage size interaction were conducted on a subset containing the 5 largest cage sizes only. Cage size effects were subsequently studied for enriched and unenriched cages separately. Data on behaviour and growth characteristics were analysed in the same way as the GCM concentrations, except that age (and its interaction with the other fixed factors) was added to the model, and that observations on the same cage were treated as repeated measures.

3. Results

Concentrations of GCM were significantly elevated after transport ($t_{83} = 8$, $P < 0.001$, Fig. 1). GCM concentrations were lower in enriched than in unenriched cages before, as well as after, transport (before transport: $F_{1,58} = 7.9$, $P = 0.007$, after transport: $F_{1,56} = 7.8$, $P = 0.007$). The response to transport did not differ significantly between unenriched and enriched cages, regardless of whether this was calculated by subtracting pre-transport GCM concentration from post-transport ones ($F_{1,52} = 0.09$, $P = 0.8$) or by dividing post-transport GCM concentrations by pre-transport ones ($F_{1,52} = 1.31$, $P = 0.3$). No significant effect of cage size or cage size × enrichment on pre- or post-transport GCM concentrations, or on the response to transport was found (all $P > 0.3$).

Significant effects of enrichment and cage size on behaviour are displayed in Table 1. Agonistic behaviour occurred in only 0.05% of the scans and visual inspection of the data did not indicate an effect of enrichment or cage size. Therefore no further analysis was performed for this type of behaviour. Comfort and locomotor behaviour were not affected by enrichment or cage size. Ingestive behaviour was decreased in 0.64 m^2 cages, but did not show a consistent pattern of increase or decrease with cage size. Resting occurred more often in the two smallest cage types than in the two largest cage types, whilst the opposite was found for stationary behaviour. Social behaviour peaked in cages of 1.07 m^2 and both social behaviour and cage manipulation occurred more frequently in unenriched than in enriched cages.

Feed intake did not differ between unenriched and enriched cages ($F_{1,52} = 2.6$ $P = 0.232$), but feed conversion was slightly better in unenriched cages than in enriched ones (Table 2). In the last week of fattening, average daily gain was slightly higher in the unenriched

Table 1
Significant effects of enrichment and cage size on behaviour. LSMEANS are displayed as percentages. Means within a row lacking a common lowercase letter differ significantly ($P < 0.05$ after sequential Bonferroni correction), as do means within a column lacking a common uppercase letter.

Behaviour	Enrichment	Cage size	LSMEANS	Cage size							Overall	SEM
				0.40	0.46	0.53	0.64	0.80	1.07	1.60		
Ingestive		$F_{6,69} = 2.5$ $P = 0.030$		12.0^{a}	10.7^{ab}	10.3^{ab}	9.5^{b}	10.6^{ab}	11.5^{a}	10.3^{ab}		0.01
Resting		$F_{6,69} = 4.0$ $P = 0.002$		65.0^{ab}	66.6^{a}	64.6^{abc}	64.4^{abc}	61.5^{bc}	60.8^{c}	61.0^{c}		0.02
Stationary		$F_{6,69} = 4.2$ $P = 0.001$		31.6^{bc}	29.8^{c}	32.4^{abc}	32.3^{abc}	35.2^{ab}	35.5^{a}	35.6^{a}		0.01
Other		$F_{6,69} = 4.1$ $P = 0.002$		69.2^{ab}	71.5^{a}	70.7^{a}	72.2^{a}	70.2^{a}	66.5^{b}	70.1^{a}		0.01
Social	$F_{1,52} = 15$ $P < 0.001$	$F_{6,39} = 3.6$ $P = 0.007$	Unenriched	2.0^{b}	1.6^{b}	1.6^{b}	2.2^{b}	2.0^{b}	3.5^{a}	2.4^{b}	2.3^{X}	0.003
		$F_{4,25} = 3.2$ $P = 0.030$	Enriched			1.1^{b}	1.5^{b}	1.4^{b}	2.4^{a}	1.5^{ab}	1.6^{Y}	0.003
Cage manipulation	$F_{1,56} = 11$ $P = 0.002$		Unenriched								2.6^{X}	0.002
			Enriched								1.8^{Y}	

Table 2
Significant effects of enrichment and cage size on growth characteristics. Means within a row lacking a common lowercase letter differ significantly ($P<0.05$ after sequential Bonferroni correction).

	Cage size	Enrichment	Enrichment × age	LSMEANS							SEM
Average daily intake per rabbit (g)	$F_{6,75}=2.6$ $P=0.026$			Cage size (m^2)	0.40	0.46	0.53	0.64	0.80	1.07	1.60	
					146.9[b]	147.0[b]	148.3[b]	149.0[ab]	149.8[ab]	152.9[ab]	155.0[a]	2.1
Feed conversion		$F_{1,52}=5.6$ $P=0.021$			Unenriched	Enriched						
					3.2[b]	3.3[a]						0.2
Average daily gain per rabbit (g)			$F_{3,1846}=2.6$ $P=0.049$		Unenriched	Enriched						
				Days 30–44	47.1	48.1						1.6
				Days 44–58	46.4	47.0						
				Days 58–63	47.5	47.2						
				Days 63–70	48.1[a]	45.3[b]						

cages than in the enriched ones. Feed intake was greater in cages of 1.60 m^2 than in those ≤ 0.53 m^2, but this did not lead to a significant effect on average daily gain or feed conversion.

4. Discussion

GCM concentrations in rabbit faeces were elevated after transport. Whilst no effect of cage size was apparent, pre-transport and post-transport GCM concentrations were lower in cages equipped with a wooden enrichment structure. Our results seem to support the theory that analysis of faecal GCM concentrations can pick up baseline differences caused by chronic stress [5] and to contradict the theory that chronic stress leads to an altered response to a novel stressor without an associated effect on baseline levels [3,5].

It is possible that our 6 week treatment period was too short to result in altered HPA-reactivity without associated effects on GCM baselines. In that case, the higher baseline GCM concentrations in unenriched cages may represent the animals' continued attempt to adapt their GC response to their environment. The notion that the rabbits were in the earlier stages of their stress response is supported by the fact that the higher baseline GCM concentrations were not accompanied by reduced weight gain, worse feed conversion or greater fearfulness [51]. On the other hand, the higher post-transport GCM concentrations for unenriched cages may indicate that the stress associated with unenriched cages had already led to a stronger response to other stressors, which is reported as an indicator of adaptation to chronic stress [3,9–11]. However, such a conclusion would only be valid if the 'response' to the novel stressor is defined by post-stressor levels, as no effect of enrichment was found on the difference between post-stressor and pre-stressor concentrations (as used by [8,12]). Possibly such post–pre differences were not found in the present study because of the long sampling interval (10 h). Such a long interval was used to maximise chances that all individual post-transport excretions peaks occurred within the sampling interval, but it may also have diluted the sample. Future research may study the effects of more prolonged exposure to enriched vs. unenriched treatments, or employ multiple shorter sampling intervals to see if a difference between post-stressor and pre-stressor values would occur at a specific part of the interval. However, the period of the present experiment covers the normal lifespan of fattening rabbits in modern husbandry systems and so reflected the real situation for rabbits kept commercially. Even though it is unclear whether the observed effects were due to being accustomed to, or getting accustomed to, barren housing conditions, rabbits kept in these conditions showed higher GCM concentrations. The higher GCM concentrations could not be attributed to a higher overall activity. However, the greater occurrence of cage manipulation and social contact in the unenriched cages may indicate that the rabbits redirected their urge to gnaw to less suitable substrates when no enrichment was provided. Since GCM concentrations were previously found to increase under circumstances that may be expected to be stressful (exposure to predator odour [19], increased predation pressure [20]), this suggests that welfare was compromised in the unenriched cages. Together with the previously reported positive effects of gnawing material on behaviour [39], this supports the call for enrichment in rabbitries. However, despite previous positive effects on production [39], enrichment did not have a favourable effect on growth characteristics in our study.

The lower baseline faecal GCM concentration in enriched, but not in larger, cages is similar to what was previously described for mink [25]. The absence of a space allowance effect is in accordance with another study in rabbits [31], and with results of faecal GCM measurement in several other species [25–27]. Nevertheless, it contrasts with another study on the effects of space allowance on rabbit GC concentrations [30], but this discrepancy may be explained by the specific selection of subordinate animals these authors made. Because the aim of our experiment was to assess GC levels of group housed rabbits non-invasively, faecal samples were collected from underneath the wire floor. Therefore samples could not be linked to individuals, and contained faeces of subordinate as well as dominant animals. These may have been influenced differently by cage size, thus adding to individual differences in GCM concentrations. An effect of stocking density on GCM concentrations has previously been described for individually sampled mice [52]. The previously described increase in fearfulness and aggression with increasing density [28,29] did not occur in our study either [51], supporting our GC data. Analysis of the rabbits' spatial distribution (Buijs et al., unpublished data) showed that the rabbits avoided each others' proximity even in the largest cages, suggesting that even these cages forced the animals in closer proximity than preferred. Thus, it is possible that the lack of a cage size effect on faecal GCM could be due to the fact that all cage sizes tested induced an increase in GCM. Alternatively, the use of relatively large group sizes (8 animals per pen) may have decreased the effects of space allowance, as larger groups have more opportunity to share space [53].

In summary, the results support the theory that measuring baseline concentrations of GCM in faeces may be a useful tool to evaluate chronic stress in rabbits and suggest that environmental enrichment may decrease housing stress in this species.

Acknowledgement

This study was made possible by financial support from the Agency for Innovation by Science and Technology, CERA and several organisations from the poultry sector. Stephanie Buijs was financed by an ILVO PhD. grant. Elin Spangenberg is thanked for her feed-back on the manuscript. Thomas Martens, Thijs Decroos and André Vermeulen and Els van Poucke are thanked for their assistance during the experiment.

References

[1] Moberg GP. Biological responses to stress: implications for animal welfare. In: Moberg GP, Mench JA, editors. The biology of animal stress. CABI Publishing; 2000. p. 1–21.

[2] Lane J. Can non-invasive glucocorticoid measures be used as reliable indicators of stress in animals? Anim Welf 2006;15:331–42.
[3] Wiepkema PR, Koolhaas JM. Stress and animal welfare. Anim Welf 1993;2: 195–218.
[4] Mendoza SP, Capitanio JP, Mason WA. Chronic social stress: studies in non-human primates. In: Moberg GP, Mench JA, editors. The biology of animal stress. CABI Publishing; 2000. p. 227–47.
[5] Mormede P, Andanson S, Auperin B, Beerda B, Guemene D, Malmkvist J, et al. Exploration of the hypothalamic-pituitary-adrenal function as a tool to evaluate animal welfare. Physiol Behav 2007;92:317–39.
[6] Hasegawa N, Nishiwaki A, Sugawara K, Ito I. The effects of social exchange between two groups of lactating primiparous heifers on milk production, dominance order, behavior and adrenocortical response. Appl Anim Behav Sci 1997;51:15–27.
[7] Ladewig J, Smidt D. Behavior, episodic secretion of cortisol, and adrenocortical reactivity in bulls subjected to tethering. Horm Behav 1989;23:344–60.
[8] Beattie VE, O'Connell NE, Kilpatrick DJ, Moss BW. Influence of environmental enrichment on welfare-related behavioural and physiological parameters in growing pigs. Anim Sci 2000;70:443–50.
[9] Harris RBS, Gu HY, Mitchell TD, Endale L, Russo M, Ryan DH. Increased glucocorticoid response to a novel stress in rats that have been restrained. Physiol Behav 2004;81:557–68.
[10] Belz EE, Kennell JS, Czambel RK, Rubin RT, Rhodes ME. Environmental enrichment lowers stress-responsive hormones in singly housed male and female rats. Pharmacol Biochem Behav 2003;76:481–6.
[11] Gupta S, Earley B, Crowe MA. Pituitary, adrenal, immune and performance responses of mature Holstein x Friesian bulls housed on slatted floors at various space allowances. Vet J 2007;173:594–604.
[12] Janssens CJJG, Helmond FA, Wiegant VM. Increased cortisol response to exogenous adrenocorticotropic hormone in chronically stressed pigs: influence of housing conditions. J Anim Sci 1994;72:1771–7.
[13] Villagra A, de la Torre JLR, Chacon G, Lainez M, Torres A, Manteca X. Stocking density and stress induction affect production and stress parameters in broiler chickens. Anim Welf 2009;18:189–97.
[14] Boiti C, Yalow RS. Corticosteroid response of rabbits and rats to exogenous ACTH. Endocr Res Commun 1978;5:21–33.
[15] Touma C, Palme R, Sachser N. Analyzing corticosterone metabolites in fecal samples of mice: a noninvasive technique to monitor stress hormones. Horm Behav 2004;45: 10–22.
[16] Szeto A, Gonzales JA, Spitzer SB, Levine JE, Zaias J, Saab PG, et al. Circulating levels of glucocorticoid hormones in WHHL and NZW rabbits: circadian cycle and response to repeated social encounter. Psychoneuroendocrinology 2004;29:861–6.
[17] Palme R, Rettenbacher S, Touma C, El-Bahr SM, Mostl E. Stress hormones in mammals and birds — comparative aspects regarding metabolism, excretion, and noninvasive measurement in fecal samples. Ann N Y Acad Sci 2005;1040:162–71.
[18] Palme R, Robia C, Messmann S, Hofer J, Mostl E. Measurement of faecal cortisol metabolites in ruminants: a non-invasive parameter of adrenocortical function. Wien Tierarztl Monatsschr 1999;86:237–41.
[19] Monclus R, Rodel HG, Palme R, Von Holst D, de Miguel J. Non-invasive measurement of the physiological stress response of wild rabbits to the odour of a predator. Chemoecology 2006;16:25–9.
[20] Monclus R, Palomares F, Tablado Z, Martinez-Fonturbel A, Palme R. Testing the threat-sensitive predator avoidance hypothesis: physiological responses and predator pressure in wild rabbits. Oecologia 2009;158:615–23.
[21] Carlstead K, Brown JL. Relationships between patterns of fecal corticoid excretion and behavior, reproduction, and environmental factors in captive black (*Diceros bicornis*) and white (*Ceratotherium simum*) rhinoceros. Zoo Biol 2005;24:215–32.
[22] Shepherdson DJ, Carlstead KC, Wielebnowski N. Cross-institutional assessment of stress responses in zoo animals using longitudinal monitoring of faecal corticoids and behaviour. Anim Welf 2004;13:S105–13.
[23] Barnett JL, Hemsworth PH, Cronin GM, Newman EA, McCallum TH, Chilton D. Effects of pen size, partial stalls and method of feeding on welfare-related behavioural and physiological responses of group-housed pigs. Appl Anim Behav Sci 1992;34:207–20.
[24] Moreira N, Brown JL, Moraes W, Swanson WF, Monteiro ELA. Effect of housing and environmental enrichment on adrenocortical activity, Behavior and reproductive Cyclicity in the female tigrina (*Leopardus tigrinus*) and margay (*Leopardus wiedii*). Zoo Biol 2007;26:441–60.
[25] Hansen SW, Malmkvist J, Palme R, Damgaard BM. Do double cages and access to occupational materials improve the welfare of farmed mink? Anim Welf 2007;16: 63–76.
[26] Buijs S, Keeling L, Rettenbacher S, Van Poucke E, Tuyttens FAM. Stocking density effects on broiler welfare: identifying sensitive ranges for different indicators. Poult Sci 2009;88:1536–43.
[27] Nicol CJ, Brown SN, Glen E, Pope SJ, Short FJ, Warriss PD, et al. Effects of stocking density, flock size and management on the welfare of laying hens in single-tier aviaries. Br Poult Sci 2006;47:135–46.
[28] Verga M, Luzi F, Carenzi C. Effects of husbandry and management systems on physiology and behaviour of farmed and laboratory rabbits. Horm Behav 2007;52: 122–9.
[29] Szendro Z, Luzi F. Group size and stocking density. In: Maertens L, Coudert P, editors. Recent advances in rabbit sciences. Melle: Institute for Agricultural and Fisheries Research; 2006. p. 121–6.
[30] Onbasilar EE, Onbasilar I. Effect of cage density and sex on growth, food utilization and some stress parameters of young rabbits. Scand J Lab Anim Sci 2007;34: 189–95.
[31] Whary M, Peper R, Borkowski G, Lawrence W, Ferguson F. The effects of group housing on the research use of the laboratory rabbit. Lab Anim 1993;27:330–41.
[32] Baumans V. Environmental enrichment for laboratory rodents and rabbits: requirements of rodents, rabbits and research. ILAR J 2005;46:162–70.
[33] Meijer MK, Sommer R, Spruijt BM, van Zutphen LFM, Baumans V. Influence of environmental enrichment and handling on the acute stress response in individually housed mice. Lab Anim 2007;41:161–73.
[34] Pohle K, Cheng HW. Comparative effects of furnished and battery cages on egg production and physiological parameters in White Leghorn hens. Poult Sci 2009;88:2042–51.
[35] Van de Weerd HA, Day JEL. A review of environmental enrichment for pigs housed in intensive housing systems. Appl Anim Behav Sci 2009;116:1–20.
[36] Spoolder HAM, Burbidge JA, Edwards SA, Simmins PH, Lawrence AB. Effect of food level and straw bedding during pregnancy on sow performance and rsponses to an ACTH challenge. Livest Prod Sci 1996;47:51–7.
[37] Dawkins MS, Edmond A, Lord A, Solomon S, Bain M. Time course of changes in egg-shell quality, faecal corticosteroids and behaviour as welfare measures in laying hens. Anim Welf 2004;13:321–7.
[38] Vasconcellos AS, Guimaraes MABV, Oliveira CA, Pizzutto CS, Ades C. Environmental enrichment for maned wolves (*Chrysocyon brachyurus*): group and individual effects. Anim Welf 2009;18:289–300.
[39] Jordan D, Luzi F, Verga M, Stuhec I. Environmental enrichment in growing rabbits. In: Maertens L, Coudert P, editors. Recent advances in rabbit sciences; 2006. p. 113–9.
[40] Lidfors L. Behavioural effects of environmental enrichment for individually caged rabbits. Appl Anim Behav Sci 1997;52:157–69.
[41] Princz Z, Orova Z, Nagy I, Jordan D, Stuhec I, Luzi F, et al. Application of gnawing sticks in rabbit housing. World Rabbit Sci 2007;15:29–36.
[42] Liste MG, Maria GA, Garcia-Belenguer S, Chacoon G, Gazzola P, Villarroel M. The effect of transport time, season and position on the truck on stress response in rabbits. World Rabbit Sci 2008;16:229–35.
[43] Palme R, Robia C, Baumgartner W, Mostl E. Transport stress in cattle as reflected by an increase in faecal cortisol metabolite concentrations. Vet Rec 2000;146:108–9.
[44] Bilko A, Altbacker V. Regular handling early in the nursing period eliminates fear responses toward human beings in wild and domestic rabbits. Dev Psychobiol 2000;36:78–87.
[45] Boonstra R, Tinnikov AA. Increased corticosteroid binding capacity of plasma albumin but not of corticosteroid-binding globulin caused by ACTH-induced changes in free fatty acid concentrations in snowshoe hares and rabbits. J Endocrinol 1998;156: 205–12.
[46] Hamilton GD, Weeks HP. Glucocorticoids in eastern cottontail rabbits (*Sylvilagus floridanus*). J Mammal 1985;66:85–8.
[47] Rehnus M, Hacklander K, Palme R. A non-invasive method for measuring glucocorticoid metabolites (GCM) in Mountain hares (*Lepus timidus*). Eur J Wildl Res 2009;55:615–20.
[48] Lepschy M, Touma C, Hruby R, Palme R. Non-invasive measurement of adrenocortical activity in male and female rats. Lab Anim 2007;41:372–87.
[49] Bosson CO, Palme R, Boonstra R. Assessment of the stress response in Columbian ground squirrels: laboratory and field validation of an enzyme immunoassay for fecal cortisol metabolites. Physiol Biochem Zool 2009;82:291–301.
[50] Touma C, Sachser N, Mostl E, Palme R. Effects of sex and time of day on metabolism and excretion of corticosterone in urine and feces of mice. Gen Comp Endocrinol 2003;130:267–78.
[51] Buijs S, Keeling LJ, Tuyttens FAM. Fearfulness in meat type rabbits at different stocking densities. Proceedings of the 16th International symposium on housing and diseases of rabbits, furbearing animals and pet animals; 2009. p. 120–5.
[52] Nicholson A, Malcolm RD, Russ PL, Cough K, Touma C, Palme R, et al. The response of C57BL/6J and BALB/cJ mice to increased housing density. J Am Assoc Lab Anim Sci 2009;48:740–53.
[53] McGlone JJ, Newby BE. Space requirements for finishing pigs in confinement: behaviour and performance while group size and space vary. Appl Anim Behav Sci 1994;39:331–8.