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Measurement of cognitive bias and cortisol levels to evaluate the effects of space restriction on captive collared peccary (Mammalia, Tayassuidae)

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Highlights

- Space-restricted collared peccaries show negative judgement bias and elevated cortisol.

- Metabolism pens may therefore compromise the welfare of collared peccaries.

- Cognitive bias paradigm is a useful tool to assess housing impact on peccaries.

Abstract

We use the judgement-bias paradigm to evaluate whether space restriction in metabolism pens affects the emotional state of collared peccary (*Pecari tajacu*) during a nutritional experiment. We trained individual adult males to 'go' to a specific location within 30s when a positive auditory cue (whistle; CS+) was given in order to receive cassava root pieces as a reward, and to 'no-go' when a negative cue (caxixi percussion instrument; CS-) was sounded to avoid punishment (jet of water) and no reward. An 'ambiguous' auditory cue (a drumstick hitting an aluminum plate; CS_A) was presented to probe decision-making under ambiguity. Individuals were subjected to five 8-day housing conditions in the order: H1 (control-no space restriction-metabolism pen and additional area), H2 (space restriction without environmental enrichment (metabolism pen only), H3 (control-no space restriction), H4 (space restriction with environmental enrichment), and H5 (control-no space restriction). On the eighth day of each housing condition, each animal was exposed to 10 judgement bias trials of each of the three cue types: CS+, CS-, and CS_A. We recorded whether animals showed the 'go' or 'no-go' response after each type of cue and collected fecal samples to assess fecal glucocorticoid metabolite concentrations. Peccaries learnt to discriminate CS+ and CS- and maintained this discrimination during the five housing conditions tested. The response to the ambiguous cue (CS_A) varied according to the housing condition. During H1, the peccaries made a similar proportion of 'go' responses to all three types of cue (Ps>0.07). During H2 and H3, 'go' responses to CS_A and CS- cues occurred in similar proportions (Ps>0.70), but peccaries showed more go responses to CS+ (Ps<0.03) indicating that they were responding to CS_A as if it were more likely to predict the waterjet than food. During H4 and H5, peccaries again made a similar proportion of 'go' responses to all three types of cue, as in H1. During H2 and H3, fecal glucocorticoid metabolite concentrations were higher than during the other tests (208.0±16.4 vs. 141.6±25.9 ng.g^{-1} dry feces, Ps<0.03). Our results suggest that space restriction may induce physiological stress and influence judgement bias and affective state in peccaries, and that these effects may be offset by environmental enrichment. However, the possibility of a general habituation to the housing conditions across time cannot be ruled out.

1. Introduction

The collared peccary (*Pecari tajacu*) inhabits Neotropical countries, and is one of the most frequently hunted species in Latin America, resulting in a population decline that threatens the species' survival (Bodmer, 2012). To avoid this, attempts to farm collared peccary are in progress in Latin American countries to supply the social and economic demands of traditional communities, which depend on wildlife as a major source of food and income (Nogueira and Nogueira-Filho, 2011). To properly farm this species, however, it is necessary to have information on its physiology and its nutritional requirements.

In order to obtain this knowledge, studies employ metabolism pens to house animals, allowing feces and urine samples to be collected so as to generate information about nutrient and foodstuff digestibility, and nutrient balances (e.g. Galagher et al., 1984; Carl and Brown, 1985). However, when maintained in the confines of a small and unfamiliar metabolism pen, collared peccary may show inactivity and lack of appetite (Mendes, 2008), perhaps reflecting a depression-like state in response to the inescapable and cramped housing conditions (cf. Hansen and Berthelsen, 2000; Petherick, 2007; Lund et al., 2012). Such a state would indicate poor welfare and may also compromise scientific outcomes, as has been suggested for other species (Hansen and Berthelsen, 2000; Petherick, 2007; Fraser, 2008; Lund et al., 2012).

To decrease such problems Nogueira-Filho et al. (2014) designed a specially modified metabolism pen for peccaries. In these pens, more space in comparison with traditional cages is supplied for peccaries to perform behaviors such as locomotion/exercise and self-grooming, alongside the ones essential for survival, such as eating, drinking, resting, and excretion. However, the welfare of peccary in such pens has not been formally evaluated. The aim of this study was, therefore, to provide such an evaluation. We hypothesized that housing peccaries in the restricted space of a metabolism pen would negatively affect their emotional state and hence

their welfare, relative to housing them in a modified metabolism pen with additional space (Nogueira-Filho et al. 2014). We also hypothesized that it is possible to minimize any detrimental effects of restricted housing in a metabolism pen by providing an environmental enrichment schedule (see Nogueira et al., 2011).

To investigate these hypotheses, we used a cognitive (judgement) bias test (Mendl et al., 2009) to assess peccary affective state. In such tests (e.g. Harding et al., 2004; Gygax, 2014), animals are trained to perform behaviour P in response to a cue predicting a positive event in order to obtain a reward, and behaviour N in response to a different cue predicting a negative event in order to avoid that event. They are then presented with occasional intermediate or ambiguous cues to investigate whether they show behaviour P indicating expectation of the reward, or behaviour N indicating anticipation of the negative event. Here we trained collared peccaries to approach a location to get food when a positive cue sounded (go response) and to keep away from the location to avoid a water jet when a negative cue sounded (no-go response). We predicted that in housing conditions that induce a relatively negative affective state, trained peccaries would respond to ambiguous sound cues as if they predicted the negative event and show no-go responses (so-called 'pessimistic' decisions), whereas peccaries in a relatively positive state would show go responses to the ambiguous cue indicating that they anticipated a reward. Our previous study of white-lipped peccaries (*Tayassu pecari*) used the same go / no-go task with acoustic cues and found results in line with these predictions when testing the effects of a simulated trapping procedure hypothesized to induce a negative affective state (Nogueira et al., 2015a).

2. Material and Methods

2.1 Ethics statement

This work followed the "Principles of laboratory animal care" (NIH publication No.86–23, revised 1985) and was approved by the Committee of Ethics for Animal Use (CEUA) at the Universidade Estadual de Santa Cruz (protocol #021/13).

2.2 Animals and facilities

We carried out an experiment with six adult male collared peccaries (*Pecari tajacu*) at the Laboratório de Etologia Aplicada, Universidade Estadual de Santa Cruz, Brazil (14° 47'39.8"S, 39°10'27.7"W). The experimental animals, aged between 4 and 6 years old and weighing 23.0±2.2 kg, were from the same herd (N=23), born and raised in captive conditions.

At the start of the 40-day experiment, these animals were transferred to 11.5 m^2 individual pens divided by a guillotine door in to two sections: metabolism pen and an additional area (Fig. 1). The metabolism pen (2.0 m x 2.0 m), with a water trough and a feeder, was entirely sheltered and with a lattice floor, which allowed feces and urine to be collected in receptacles located below. The additional area comprised a partially sheltered section (2.0 m x 2.5 m) and an unsheltered 2.2m^2 'solarium' section with a cement floor (Fig. 1). We covered the 1.5m-high wire-fences of the pen with a white canvas to block the animals' view of neighboring pens so as to minimize visual interference from animals in adjacent pens. At the moment of introduction into the pens, the animals were weighed and dewormed (fenbendazole; 5 mg/kg of live weight). Animals were fed once a day (at 0800 h) with a diet containing corn, soybean meal, mineral salts, and vitamin supplements, with 140 g crude protein/kg and 16.7 MJ gross energy/kg (Nogueira-Filho, 2005); water was available *ad libitum*. During the first week, we determined the animals' feed intake by calculating the difference between the amount provided and the amount left over the next day. After that and throughout the entire experiment, we restricted food to 0.9 of the lowest feed intake recorded, equating to 0.7 kg of the experimental diet per day, in order to ensure that animals were motivated to eat cassava root slices (a favoured foodstuff, Nogueira-Filho, unpublished data), which were used as a reward during training and judgement-bias tests.

2.3 Animal training

At the start of each judgement bias training and test trial, animals were located in the solarium area (Fig. 1). We individually trained peccaries to 'go' to a location in the metabolism pen where a reward was provided in a food bowl (Fig. 1) when a positive auditory cue was sounded

by the observer assistant, standing behind the front wall of the metabolism pen; the positive conditioned stimulus was a 3s whistle: CS+, $Freq_{average}$3kHz; 110.1dB. We measured all-sound pressures at the food bowl location about 1m from the sound sources. If the peccary reached the reward site within 30s it received 20g of cassava root slices. We then trained the animals to 'no-go' to the same location when a different 3s auditory cue was sounded (caxixi, a percussion instrument: CS-, $Freq_{average}$ 9kHz; 80.4dB) in order to avoid a negative stimulus – a waterjet – which was delivered if they moved to the reward site (the empty food bowl) within 30 sec. To reach the reward site after hearing the cue, the individuals had to move from the solarium area through a door and into the metabolism pen (Fig. 1). Most judgement bias studies employ cues that vary along a uni-dimensional sensory scale (e.g. tones differing in frequency), hence allowing ambiguous cues with intermediate properties to be used. However, following Douglas et al. (2012) who found it difficult to train domestic pigs to discriminate a graded series of tones within a limited time window, we trained white-lipped peccary (Nogueira et al., 2015a) using categorically distinct sounds (whistle, horn, and bell) as positive, negative and ambiguous cues, and found that they did respond to the 'ambiguous' cue by showing an intermediate response indicating that they perceived it as providing uncertain information as to whether to make a 'go' or 'no-go' decision (Nogueira et al., 2015a). Given the absence of a suitable tone generator at the research site, and the success of this approach in training a closely-related wild species on the task, we used the same approach here with collared peccary.

Training on the judgement bias task involved exposing peccary to 10 CS+ training trial per day for 12 consecutive days, totaling 120 trials, and 10 CS- training trials per day for just three consecutive days, because animals learned the 'no-go' response to the CS- faster than the 'go' response to the CS+. All animals were trained individually and we completed this phase of training when animals achieved a learning criterion of at least 70% correct responses in all training trials (CS+ and CS-). We then exposed peccaries to nine further training sessions, one session per day, each containing a mix of five CS+ and five CS- cues presented in a randomly determined order (a total of 45 CS+ and 45 CS- trials per animal).

During the whole experiment (training sessions, housing treatments, and judgement-bias tests), the animals remained in the same pen. The door between the metabolism pen and the additional area was opened or closed to provide more or less space for the animal, and a simple environmental enrichment schedule was included (see below).

2.4 Housing treatments and the judgement-bias test procedure

Following successful training, the peccary were exposed to housing treatments. Each experimental housing condition (H1, H2, H3, H4, and H5, detailed below) lasted eight consecutive days and nights, and the judgment bias trial tests occurred on the morning and late afternoon of the eighth day of each condition (Fig. 2). The five experimental conditions and accompanying judgement bias tests were implemented in succession such that the experiment ran for 40 consecutive days (Fig. 2). The five housing conditions were, in order: H1 (control-no space restriction), H2 (space restriction without environmental enrichment), H3 (control-no space restriction), H4 (space restriction with environmental enrichment), H5 (control-no space restriction) (Fig. 2). During control conditions (H1, H3, and H5) the animals could freely move around the entire pen (metabolism pen, sheltered area and solarium), while during both space restriction conditions (H2 and H4) the animals were restricted to the metabolism pen only (Fig. 1). During H4 we introduced two coconuts inside the metabolism pens as temporally unpredictable environmental enrichment, following Nogueira et al. (2011), who showed that temporal unpredictability increases the exploratory behavior and activity of peccaries. The animals could not open or eat the coconut, which would have affected the nutrition results; they just rolled it along the pen floor. We introduced the coconuts inside the pen and left them for one hour per day at a moment randomly determined between 1000 and 1500 h. During H2, the animals were also contained inside the metabolism pen but without the environmental enrichment. At the end of the experimental period, we released the animals into their original enclosure.

In judgement bias tests a 3s 'ambiguous' auditory cue – a drumstick hitting an aluminum plate, CS_A (Freq$_{average}$ 6 kHz; 62.8dB) was presented to the animals. The empty food bowl was in the same position as during training (Fig. 1). 'Go' or 'no-go' decisions were not rewarded or punished. We presented this cue to probe decision-making under ambiguity and to investigate whether animals responded to this cue as if predicting the reward – 'optimistic' response – or punishment – 'pessimistic' response (Harding et al., 2004).

We carried out judgement bias tests on the morning and late afternoon of the eighth day of each housing condition when the animals were most active (from 0700 until 0930 and from 1530 until 1800). The judgement bias test for housing conditions H2 and H4 started in the morning just after Area A (Fig. 1) was opened. At the end of the tests, the peccaries were again restricted to the metabolism cage (Area B, Fig. 1) until the afternoon test when Area A was opened once more. Following these tests, peccary had access to both areas open for the H3 and H5 (control-no space restriction) housing conditions. Each test comprised 10 trials of each of the three cues: CS+, CS-, and CS_A (i.e. 30 trials per test and 150 trials in total across tests T1–T5). Trial order (CS+, CS- or CS_A) was randomly determined by the drawing of lots. On each trial, we recorded whether the animal reached the food bowl- ('go' response) within 30s, or whether it remained at least 1m from the reward location for 30s ('no-go' response). Animals were returned to a start location prior to the next trial by using a voice command. After an interval of 15min, we carried out the same procedure with the next peccary. Peccary test order was randomly determined. We recorded the animals' responses by using a digital camcorder (JVCGZ-HD500; Tokyo, Japan).

After the end of afternoon trials, we collected fecal samples to determine the concentration of fecal glucocorticoid metabolites. We packed the samples in marked plastic containers and refrigerated them at -20∘C (Coradello et al., 2012). The fecal samples were homogenized and 1–2g from each individual animal was sub-sampled and stored at −20ºC in preparation for freeze-drying (FreeZone® Plus 4.5 Liter Cascade Benchtop, LABCONCO) following Wasser et al. (2000). The concentration (ng/g) of the glucocorticoid metabolites in

these freeze-dried samples was determined at the Laboratório de Medidas Hormonais of the Universidade Federal do Rio Grande do Norte, Brazil. Procedures used to extract and to assay fecal glucocorticoid metabolite concentrations through ELISA followed Möstl and Palme (2002) and Brown et al. (2004). However, instead of the usual dilution at 1:50 in ethanol, we used 1:10 for best recovery of glucocorticoid metabolites as determined by Coradello et al. (2012). Intra and inter-assay coefficients of variation were 2.8±0.2% and 9.8±2.3%, respectively, validating the assay's precision (Brown et al., 2004; Möstl et al., 2005). The antibody had the following cross-reactivities: cortisol 100%, prednisolone 9.9%, prednisone 6.3%, and cortisone 5%. The limit of detection was 29.9 ng/g of dry feces. Also called assay sensitivity, this limit of detection was calculated by subtracting two standard deviations from the mean counts in 15 samples obtained during control tests.

2.5 Statistical analyses

To determine if animals had learnt the discrimination task during the final nine days of training, we ran Mantel-Haenszel Chi-square tests on combined data from five out six animals for each day (i.e. for 25 CS+ and 25 CS- trials) of the nine training sessions (probability of correct response on each trial = 0.5). Animal 6 showed only 40% accuracy in their responses to both the CS+ (go) and CS- (no-go) cues during the final nine days of training, indicating that it did not learn the discrimination task. Therefore, this animal was not used in this analysis or subsequent test sessions. The Mantel-Haenszel Chi-square tests were performed with the CHISQ and PROC FREQ options of SAS (version 8.02, 2001; Statistical Analysis Systems, Cary, NC).

For the five test sessions, we calculated the proportion of trials in which each animal reached the food bowl within 30s ('go' response) for each cue type. We analyzed data using a GLM with repeated measures followed by post-hoc Duncan tests. Housing type (H1, H2, H3, H4, and H5) and cue type (CS+, CS-, CS_A) were within-subject factors and we examined their effects, including interactions, on the proportion of 'go' responses made. We used post-hoc tests

where appropriate to investigate differences between responses to all three cue types at each test point, hence taking into account any drift in 'baseline' responses to CS+ and CS- across tests. We analyzed the mean concentration of fecal glucocorticoid metabolites using a similar statistical model incorporating only test (housing) type (H1-H5) as a within-subjects factor. Data fulfilled parametric requirements of normality of residuals and homogeneity of variance and are presented as mean±S.D. The GLM analyses were performed using Statistica version 7.0 (StatSoft, Tulsa, OK, USA). In all statistical tests, a P value<0.05 was considered significant.

3. Results

The number of 'go' responses that peccaries made to the CS+ cue and no-go responses that they made to the CS- cue increased from the first to last of the nine final days of training (Fig. 3). From day 4 onwards peccaries showed significantly more correct than incorrect responses to both the CS+ ('go') and CS- ('no-go') cues (Ps<0.04, Fig. 3), indicating that they had learnt the discrimination task.

There was no main effect of housing type on peccaries responses ($F_{4, 16} = 0.21$, P = 0.93). There was, however, a clear effect of cue type ($F_{2, 8} = 18.18$, P = 0.001) and an interaction between cue and housing types ($F_{8, 32} = 2.77$, P = 0.02). Post-hoc tests showed no significant change in CS+ (Ps>0.35, Fig. 4) or CS- (Ps>0.39, Fig. 4) across the five tests. In all tests, the collared peccaries were more likely to approach the rewarded location after the positive cue than after the negative cue (Fig. 4). Responses to the ambiguous cue (CS_A) varied according to the housing type.

During the first control test (H1), the proportion of 'go' responses to the CS_A cue (0.30±0.40) was similar (Ps>0.07) to that made to the CS+ (0.50±0.16) and CS- (0.22±0.33) cues (Fig. 4). During the two subsequent tests – space restriction without environmental enrichment (H2) test and the following control condition (H3) test – 'go' responses to CS_A (0.26±0.30) and CS- (0.21±0.28) cues occurred in similar proportions (Ps>0.70), but were less common than 'go' responses to the CS+ cue (0.44±0.37; Ps<0.03). Thus peccaries appeared to respond to the ambiguous cue as if it were more similar to the negative cue during these tests (a

'pessimistic' bias). During the space restriction with environmental enrichment test (H4) as well as during the last control test (H5), the proportion of 'go' responses to the CS_A cue (0.37 ± 0.34) were similar ($Ps>0.25$) to that made to the CS+ (0.45 ± 0.37) and CS- (0.28 ± 0.34) cues (Fig. 4). Thus, as in the first control test (H1), peccaries showed neither an 'optimistic' bias nor 'pessimistic' bias in how they responded to the ambiguous cue relative to the training cues.

Test housing type affected fecal glucocorticoid metabolite levels ($F_{4, 16} = 8.93$, $P = 0.0005$), with post-hoc tests indicating that concentrations were higher following H2 and H3 housing treatments than following the other types of housing ($Ps<0.03$, Fig. 5). During all the five housing conditions tested, we recorded no variation on feed intake and an equal total feed intake of the experimental diet – 0.7 kg per day.

4. Discussion

We found that the peccaries did not show a biased judgement of CS_A following the first and last control housing periods (H1 and H5) and after the housing with space restriction and environmental enrichment (H4). In contrast, during the judgement bias tests on day 8 of space-restricted housing (H2) and the subsequent non-restricted control period (H3), collared peccaries' made responses to CS_A that were more similar to their responses to the negative than the positive training cues. This suggests that they were anticipating a higher likelihood of a punishing than a rewarding outcome under ambiguity (a 'pessimistic' response) during both H2 and H3. We also found higher fecal glucocorticoid metabolite concentrations following these two housing conditions in comparison to the other housing treatments.

Overall, and following predictions relating decision-making under ambiguity to affective state (Paul et al., 2005; Mendl et al., 2010), the results indicate that peccaries may have experienced a relatively negative affective state during tests after conditions H2 and H3 compared to tests after conditions H1, H4, and H5. This suggests that restricting housing space (H2) has negative effects on peccaries, and that this may be ameliorated by environmental enrichment (H4). However, it is also possible that, over time, the peccaries adapted to the

restricted space and hence were less affected by space restriction *per se* in H4 compared to H2. Due to experimental and time limitations, however, we could not test variation in housing condition order across subjects, and the Committee of Ethics for Animal Use (CEUA) at the Universidade Estadual de Santa Cruz did not allow the experiment to be extended to do further studies using a counter-balanced order. Further study is needed to test the effects of variation in housing condition order across subjects so as to elucidate whether the results were primarily caused by changes in the housing *per se* or habituation.

Why a negative judgement bias was also observed during the H3 control period following the H2 housing is not entirely clear. One possibility is that the effects of the H2 housing conditions took longer than a week to dissipate. The elevated levels of fecal glucocorticoid metabolites found during both the H2 and H3 conditions are consistent with the judgement bias findings, if we assume that they reflect a relatively long-lasting negatively valenced physiological stress state induced by confinement during H2. This seems a reasonable assumption given that adverse conditions activate the adrenal gland and cause an increase in glucocorticoids (Möstl and Palme, 2002) as a natural adaptive response to perceived threat (Sapolsky, 1982), but an unequivocal conclusion is precluded by the knowledge that elevated glucocorticoids can also occur in conditions of positively-valenced affective arousal (e.g. Buwulda et al., 2012).

Post-hoc tests showed that during all five judgement bias tests, peccaries responded differently to CS+ and CS- cues, and we also recorded no significant changes in CS+ or CS- responses across tests. These findings indicate that the peccaries continued to discriminate between cues predicting reward and punishment, including during space restriction housing conditions. This indicates that animals learnt the discrimination task, and that the cassava root slices and waterjet worked as effective reinforcers for peccaries.

Responses to CS_A cues were usually intermediate between those to CS+ and CS- cues suggesting that, despite the use of categorically distinct sounds, peccaries appeared to treat the

ambiguous cues as providing uncertain information as to whether a reward or punishment was to follow, as is the case when intermediate cues on a unidimensional sensory scale are used. Similar findings were reported by Douglas et al. (2012) and Nogueira et al. (2015a). Furthermore, the post-hoc tests showed that peccaries' responses to the ambiguous cue in the last control test (H5) did not differ significantly from those seen in the initial control test (H1). This indicates that the animals did not learn across trials that this cue was not rewarded, as has been suggested in some other studies (e.g. Brilot et al., 2010; Doyle et al., 2010; Murphy et al., 2013), and hence that any increases in 'no-go' responses to the ambiguous cue relative to responses to the CS+ and CS- cues (as in tests H2 and H3) were unlikely to have been due to extinction of a 'go' response to CS_A.

In comparison with previous studies, fecal glucocorticoid metabolite concentration throughout the different housing conditions was higher than the species' basal levels of 29.7 ± 11.2 ng/g dry feces (Coradello et al., 2012). This can probably be explained by the stress resulting from social isolation in the present study due to the canvas placed between pens. Collared peccaries are social animals that live in herds ranging from six to 30 animals (Sowls, 1997). Thus, during nutritional experiments, we recommend that peccaries caught from the same herd should be kept in neighboring pens separated just by wire fencing, which allow some body contact as well as visual. Finally, when maintained inside the specially designed metabolism pens, the collared peccaries consumed all of the (restricted) rations offered, contrary to the lack of appetite recorded by Mendes (2008), in a study that restrained peccaries in smaller metabolism cages. Therefore, the experimental condition used here - the specially designed metabolism pens - did not affect feed intake of collared peccaries.

Overall, our findings suggest that negative judgements of ambiguous cues in the cognitive bias paradigm may reflect housing-induced negative affect in captive collared peccaries. A previous study using the cognitive bias paradigm in white-lipped peccary (*Tayassu pecari*) also detected a negative judgement of ambiguity following a putatively stressful trapping event (Nogueira et al., 2015a). Together, these studies suggest that decision-making

under ambiguity may be a useful indicator of affective state, and hence welfare, in both white-lipped and collared peccary, and following both short- and longer-term challenges.

5. Conclusions

Our results indicate that space restriction may compromise the welfare of collared peccaries, leading to a more cautious 'pessimistic' response to ambiguous stimuli associated with cortisol increase. It also appears that environmental enrichment might help to mitigate detrimental effects of space restriction, although an additional or alternative influence of habituation to space restriction cannot be ruled out. Overall, the study indicates that the cognitive bias paradigm is a useful tool for assessing the impact of housing conditions on peccaries and their welfare.

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Figure's Captions

Fig. 1. Diagram of an experimental metabolism pen.

Fig. 2. The timeline of the experimental design.

Fig. 3. Number of correct and incorrect responses made by animals (N=5) during the final nine training sessions. Significance levels show the results of Mantel-Haenszel Chi-square tests with a probability of 0.5 for success on each trial.
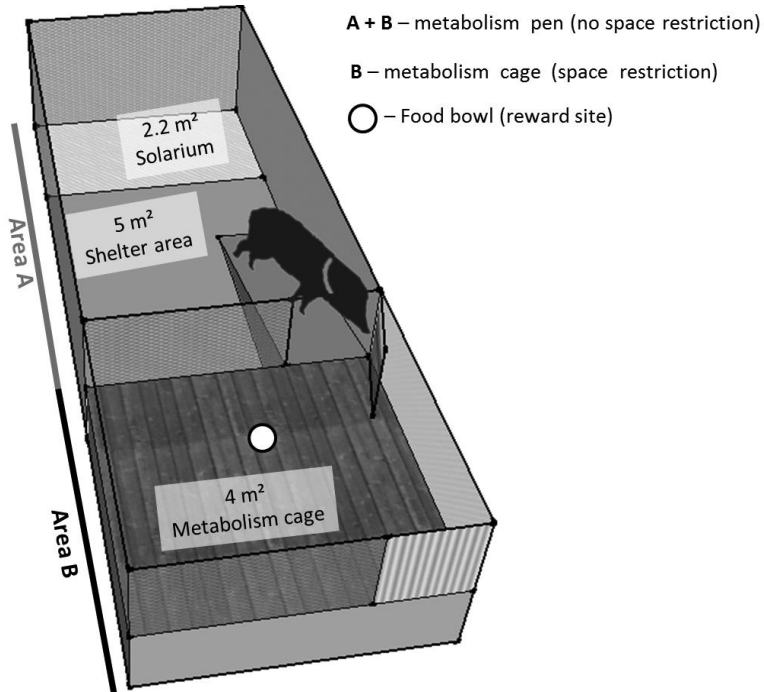
Fig. 4. Mean (+SE) proportion of 'go' responses after cues throughout the housing conditions. H1 (control-no space restriction), H2 (space restriction without environmental enrichment), H3 (control-no space restriction), H4 (space restriction with environmental enrichment), and H5 (control-no space restriction). CS+ positive auditory cue; CS- negative auditory cue; CS_A 'ambiguous' auditory cue. Within and between each test type, bars with different superscripts are significantly different according to post-hoc tests.

Fig. 5. Mean (+SE) fecal glucocorticoid metabolite concentration throughout the housing conditions. H1 (control-no space restriction), H2 (space restriction without environmental enrichment), H3 (control-no space restriction), H4 (space restriction with environmental enrichment), and H5 (control-no space restriction). Bars with different superscripts are significantly different according to post-hoc tests.
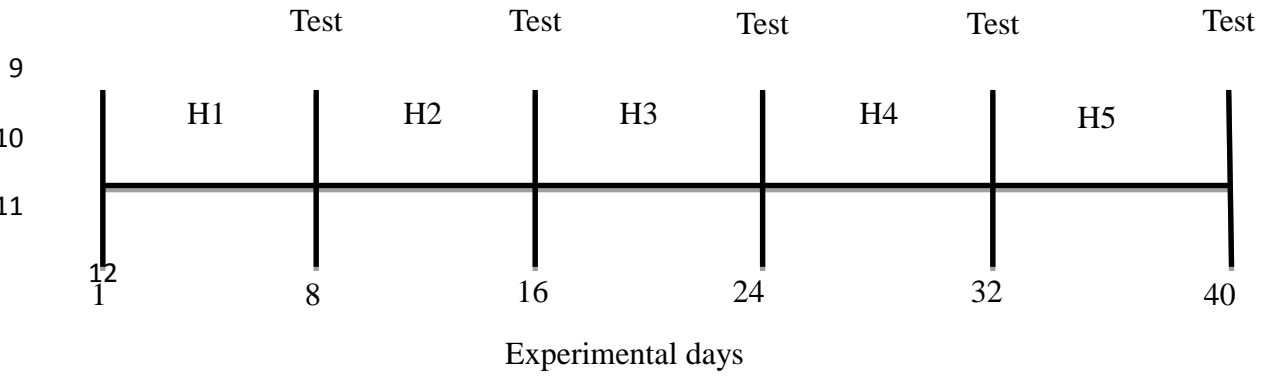
1   Figure 1.

2

3

A + B – metabolism pen (no space restriction)

B – metabolism cage (space restriction)

◯ – Food bowl (reward site)

2.2 m²
Solarium

Area A

5 m²
Shelter area

Area B

4 m²
Metabolism cage

4

5

6

Figure 2

Experimental days
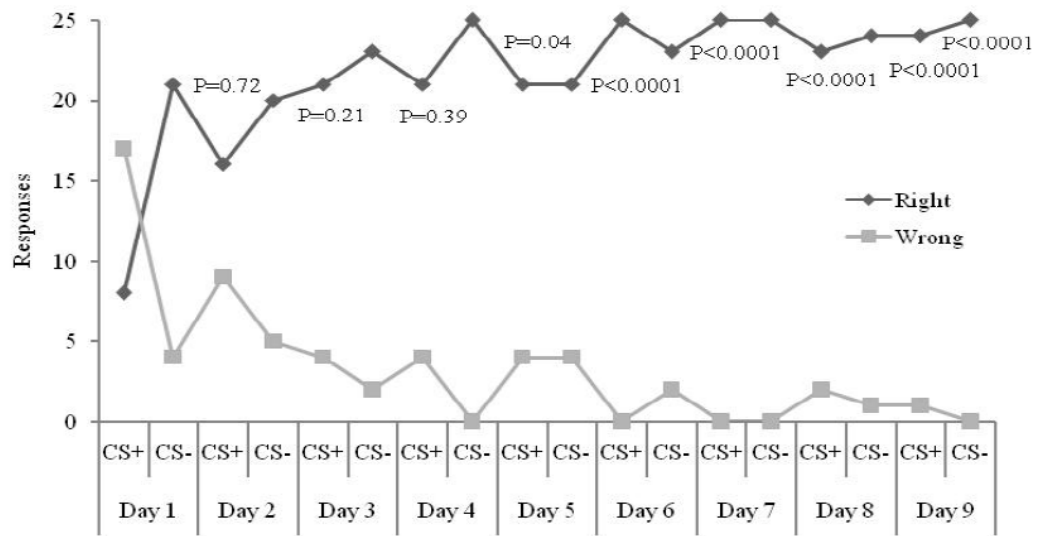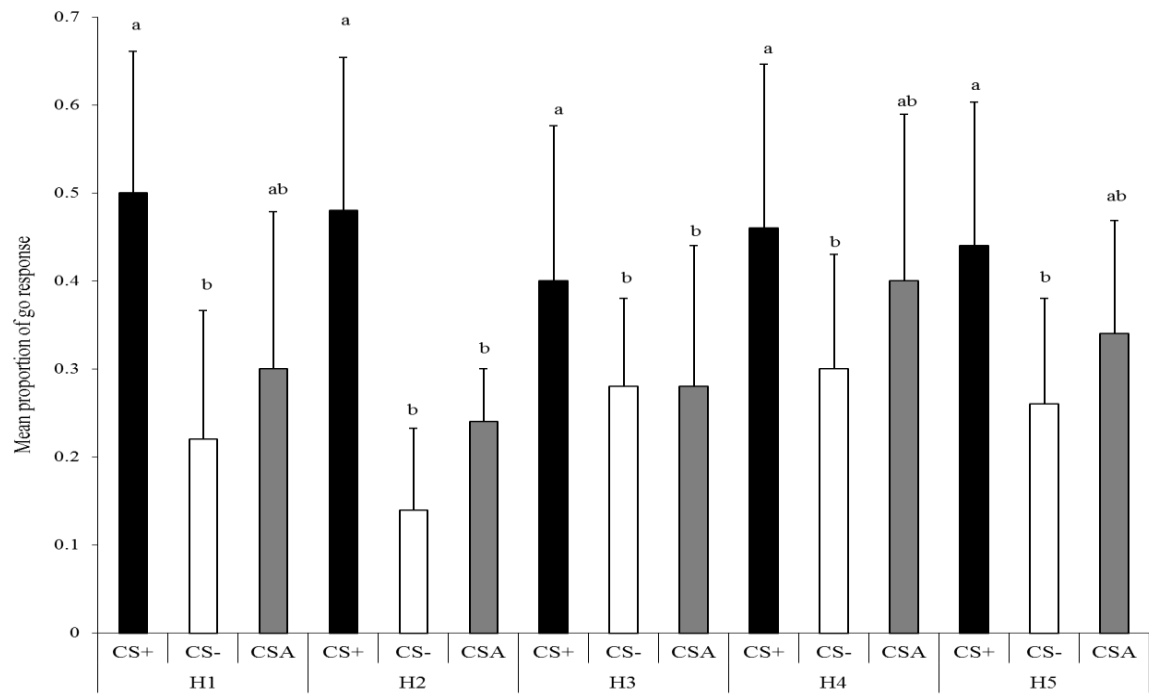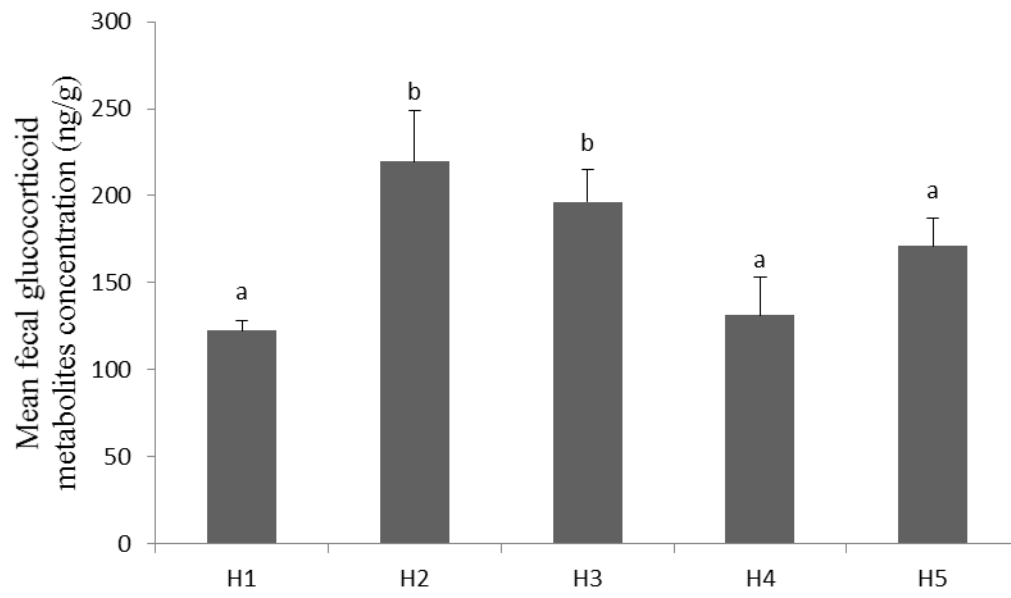
15   Figure 3

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23   Figure 5

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