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**LAYWEL**

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Revision [final]

## **Task 4.3 part 2. Final version**

### **Substrate preferences in chickens selected for and against feather pecking behaviour**

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## **Abstract**

It is not known whether birds differing in genetic susceptibility for feather pecking also differ in their preferences for various substrates. Therefore, the objective of the present study was to investigate relative preferences for specific substrates in laying hens selected for high (HFP) or low (LFP) feather pecking. First, during rearing bird preference for either peat moss, straw or sand was tested in a situation in which the birds could freely move between the home pen (littered with wood shavings) and different choice pens with one of the three test substrates. HFP pullets spent more time in straw as compared to LFP and control birds, whereas HFP birds spent less time in peat as compared to LFP and control birds. The lines did not differ in preference for sand. Second, during laying the relative substrate preference of the birds was tested using the consumer demand approach. Part of the birds (eight LFP and eight HFP birds) had to push a weighted door to enter either a wire floor, or sand, wood shavings or peat moss as substrate. The results of the preference test during rearing could not be confirmed using the consumer demand approach during laying. HFP birds did not have a higher preference for straw as compared to LFP birds. LFP birds seemed to have a higher preference for wire at lower weights as compared to HFP birds, whereas HFP birds seemed to have a higher preference for peat moss to take a dustbath. However, these results should be interpreted with care due to the small number of birds working for a dustbath. In conclusion, results of the preference test during rearing suggest that birds differing in genetic susceptibility for feather pecking also differ in their preference for substrates, and that HFP pullets prefer straw. It is not clear yet if these substrate preferences persist during laying, as no differences in substrate preference were found between LFP and HFP birds using the consumer demand approach during the laying period.

## 1. Introduction

Feather pecking in chickens has been suggested to originate from an erroneous perception of stimuli resulting in a redirection of foraging or dustbathing behaviour (e.g., Blokhuis *et al.*, 1989; Vestergaard *et al.*, 1993). A range of substrates have been investigated in relation to preventing feather pecking, and material that can be manipulated, such as straw, seems to be superior to substrates that can only be pecked and swallowed, such as sand (Huber-Eicher *et al.*, 1997, 1998; Aerni *et al.*, 2000). It is not known whether birds differing in genetic susceptibility for feather pecking also differ in their preferences for various substrates. If so, this might explain occurrence of feather pecking even in ‘optimal’ housing conditions because these conditions may be optimal for some birds or genotypes, but not for others. The objective of the present study was to investigate relative preferences for specific substrates in laying hens selected for high (HFP) or low (LFP) feather pecking (Kjaer *et al.*, 1997; Kjaer *et al.*, 2001).

Preference tests have been frequently used to study relative preference for environmental resources in chickens (e.g., Dawkins, 1981; Bradshaw, 1992; Widowski *et al.*, 1992), but more recently the consumer demand approach has become more popular to study environmental preferences (Dawkins, 1983; Matthews *et al.*, 1995; Cooper *et al.*, 1996; Gunnarsson *et al.*, 2000; Cooper *et al.*, 2003; Cooper, 2004; de Jong *et al.*, 2005). Whereas in a preference test the animal can move freely between the home pen and different choice pens, using the consumer demand approach the animal has to work to enter a specific choice pen. The underlying idea is that the animal in this way defends consumption of important resources but not consumption of luxuries (e.g., Cooper, 2004).

In the present experiment we investigated the relative preference for different substrates (peat moss, straw and sand) in laying hens selected for or against feather pecking

behaviour, using both experimental approaches. First, during rearing, the birds were subjected to a preference test. Pullets could move freely between the home pen and three different choice pens with either peat moss, straw or wood shavings. Thereafter, part of the birds used in the first experiment were transported to another experimental facility where the relative choices of the birds were determined using the consumer demand approach. In this experiment, hens had to push a weighted door to enter one of four choice pens with either a wire floor, or sand, peat moss or straw as substrate.

## **2. Materials and Methods**

### *2.1 Birds*

Three experimental lines of White Leghorn laying hens were used (Kjaer *et al.*, 2001), among which line LFP was selected for low feather pecking, line HFP for high feather pecking, and line CON was a random mating control line. The three lines were established in 1995 and derived from a White Leghorn layer line, which was formed in 1970. Feather pecking behaviour was measured at about 30 wk of age, by counting feather pecks (FP pecks) and then grouping pecks into bouts (events, feather pecking bouts, FP bouts). One bout was defined as a series of continuous pecking direct to the same individual. Selection was based on number of FP bouts.

### *2.2 Experiment 1: preference test*

#### 2.2.1 Housing and management

The experiment was conducted with pullets of the 7th generation of selection. A number of 180 chickens (60 from each of three lines, i.e. LFP, HFP and CON (control) line)

were used in each of two independent replicated experiments (hatches) three weeks apart. The experiments were conducted in a climate controlled house. Temperature was 34°C at day old and reduced gradually to 20°C at 8 weeks of age. This temperature was kept throughout the rest of the experiment. Chickens were placed from day old in a 2 by 6 m pen littered with wood shavings.

From 5 weeks of age access was given to three choice pens each measuring 2 by 2 m and littered with wood shavings. From 11 weeks onwards the choice pens were littered with straw, sand and peat, respectively. After 2 weeks the litter was changed between pens until each substrate had been presented in each choice pen. The light programme was 12L:12D with the light turning on at 06:00 h GMT+2. Light intensity was between 5 to 10 lux during the light period and below 1 in the dark period. The facility was not totally light proof. Enough light was let in to facilitate the birds to move around in the dark phase.

### 2.2.2 Transponders and antennas

All birds were fitted with a leg band carrying an electronic transponder (approx. 0.5 g, 2 mm in diameter and 1 cm long emitting a unique number when close to an antenna). Circular antennas (diameter=15 cm) and computer programs for scanning were custom built (Paratek, DK-5230 Odense, Denmark). A total of 24 antennas per pen were used. They were placed under the litter and covered all accessible area in a grid with one antenna per square m. The system scanned each antenna every 30 s and stored information for bird identity, antenna location, date and time of day. The time spent at a certain antenna was estimated to 30 s for each time it was scanned, the minimum time spent at an antenna being 30 s. Data was stored in ascii-files for each day. Scanning was done continuously round the clock for 7 days in each of three weeks when birds were 12, 14 and 16 weeks of age.

### 2.2.3 Statistical analyses

The data on relative use of the various substrates were not normal and transformations could not yield normality. A generalized linear model with gamma variance distribution and log link was used to test models, calculate estimates and contrasts. SAS software (Proc Genmod; SAS Inst., 1996) was used.

## 2.3. *Experiment 2: consumer demand approach*

### 2.3.1. Animals and Housing

The experiment was conducted with pullets of the 7<sup>th</sup> generation of selection. Sixteen laying hens (eight HFP birds and eight LFP birds) aged 16 weeks at arrival at the experimental farm were used. Hens were reared at the Danish Institute for Animal Sciences as described in 2.2.1. and transported to The Netherlands at 16 weeks of age. In addition to the test birds, 27 companion birds (13 LFP birds, 19 HFP birds) were obtained from the same rearing farm and rearing group. All birds were wingtagged and housed in groups of three or four birds per line in pens (0.75 x 1.0 m) with wood shavings and four laying nests. Test hens were subsequently tested in batches of four birds, thus, in total four batches were tested. The first batch of hens was tested at 25 weeks of age and the subsequent batches at 28, 31 and 34 weeks of age respectively. Three weeks before the start of the test all hens were weighed (mean weight 1124 g) and hens of one batch were randomly chosen from four different pens and individually housed in a pen with substrate (0.75 x 1.0 m) and a laying nest. In this way hens were given the possibility to habituate to individual housing and to the different substrates (by housing them one week in each of the substrates, i.e. chopped straw, peat moss and sand). Sequence of substrates was randomised per hen. After this habituation period, the

test period started. All hens were in lay during testing. Food and water were always available *ad libitum*. Lights were on from 02.00 h – 18.00 h GMT+2.

### 2.3.2. Test Arena

Figure 1 shows a schematic drawing of the test arena. The test arena consists of a home pen and four choice pens. From the home pen hens could reach one of the choice pens through a one-way vertically swinging door ('push door') that was based on a design of earlier studies (Petherick *et al.*, 1990; Cooper *et al.*, 1995; Olsson *et al.*, 2002a; Olsson *et al.*, 2002b; Cooper *et al.*, 2003). The choice pens had either a wire floor or a 5 cm layer of peat moss, sand or straw as substrate. Water and a nest box were available in the home pen (wire floor), a filled feed trough was available in all choice pens and the home pen. Entrance doors to the choice compartments were weighted whereas exit doors were always unloaded. Weights were attached to the door via a pulley. When the door was unloaded only a light force at shoulder height was necessary to open the door.

Because previous experiments showed that hens in isolation performed less in the test pen (i.e. learning the push door was more difficult) (De Jong, unpublished results), companion hens were housed in the rooms in such a way that at each corner of the test pen a test hen could have visual contact with a companion hen. Location of the different substrates in the test pen was changed after a complete test session of one hen was finished.

### 2.3.3. Training and test protocol

The total test procedure lasted 21 days. Each hen started with the push doors open for 48 h, to facilitate exploration of the choice pens. Thereafter doors were closed and the following weights were attached to the doors for 48 h: day 3, 4: 0 g (0 N), day 5, 6: 50 g (0.29 N), day 7,8: 100 g (0.59 N), day 9,10: 150 g (0.88 N), day 11, 12: 250 g (1.47 N), day 13, 14:



500 g (2.94 N), day 15, 16: 750 g (4.41 N), day 17, 18: 1000 g (5.88 N), day 19, 20: 1250 g (7.35 N). Cameras were mounted above all test arenas; behaviour of the hens in the test pen was videotaped continuously using time-lapse video recording.

#### 2.3.4. Recordings from the videotapes

The behaviour of the hens during the light period of two subsequent days with the following weights was recorded: 150 g, 250 g, 500 g, 750 g, 1000 g, 1250 g. In a previous experiment (de Jong *et al.*, 2005) it was found that between 0-100 g the hens were learning the push door whereas from 150 g onwards it seemed that the hens learned precisely how to push the door. We therefore analysed the behaviour of the hens from 150 g onwards. The location of the hens, the time in a particular choice pen or in the home pen and dustbathing behaviour were scored continuously during the light period using the Observer software (version 4.1., Noldus, Wageningen, The Netherlands). The period from 07.00 – 09.00 h was excluded from the analysis because in this period the caretakers were in the rooms and the weights were changed. The dustbathing behaviour was considered to begin when a hen squatted down and performed vertical wing shaking. The end of a dustbath was determined by the start of an interval of more than 15 min that did not include dustbathing behaviour. Sequences that included a shorter interval without dustbathing were considered to belong to one uninterrupted dustbath (Van Liere *et al.*, 1990).

In addition to the slopes of the demand curves (price elasticity) (e.g., Cooper *et al.*, 2000; Mason *et al.*, 2001; Cooper, 2004) calculated from the (behavioural) observations, we scored the maximum weight pushed to visit a choice pen or to visit a choice pen to perform dustbathing or foraging ('reservation price' (Cooper *et al.*, 2000; Mason *et al.*, 2001), the 'total expenditure' which was defined as the sum of the number of visits per weight category

x weight pushed per visit (Mason *et al.*, 2001) and the consumption, i.e. the time spent dustbathing as percentage of the total time in a particular choice pen (Cooper *et al.*, 2000).

### 2.3.5. Statistical Analysis

Per response variable, per individual, observations were regressed on (natural) logarithms of weight (as attached to the door). The regression coefficient (slope) was saved as an overall measure of trend. For percentages, the empirical logit transformation was applied to the observations prior to regression:

$$y = \log\left(\frac{\textit{percentage} + 0.5}{100 - \textit{percentage} + 0.5}\right).$$

Count data were log transformed prior to regression:

$$y = \log(\textit{count} + 1).$$

The slope was analysed as a new response variable. Per substrate, lines were compared by the Mann-Whitney test (Wilcoxon's two sample test) (Conover, 1980).

Substrates were compared pairwise. Differences between slopes of the same animal for two different substrates were compared by (1) Wilcoxon's signed rank test (Conover, 1980) as a test for a main effect of substrate (on slope) over lines, (2) by the same test, but separately for each line and (3) between lines with the Mann-Whitney test as a test for interaction between substrates and lines. The following variables were introduced:

TP = total weight pushed by an animal (total price),

MP = maximum weight pushed by an animal (maximum price),

HP = weight (obtained by linear interpolation) where half of the total weight is pushed,

RP = total price for a substrate as a percentage of the total weight pushed by an animal over all substrates.

HP is an alternative for MP that is expected to be more stable. RP can be seen as an alternative version of TP corrected for the willingness of an animal to push weight. It indicates the preference of the animal relative to the other substrates. Variables TP, MP, HP and RP were analysed in the same way as described before for the regression coefficient. In addition TP, MP, HP and RP were analysed by a linear mixed model (Searle *et al.*, 1992) comprising random animal effects and main effects and interaction for lines and substrates. The mixed model analysis comprised all observations for all substrates. The random animal effects accounted for the correlation between repeated measurements on the same animal. Substrate effects were tested by the Wald-test and substrates were compared pairwise by Fisher's LSD method.

All calculations were performed with the statistical software package GenStat (Committee Genstat, 2000).

### **3. Results**

#### *3.1. Experiment 1: preference test*

A total of 64,904 data points were collected comprising a total of 1,036 hours of transponder recordings. Selection for feather pecking changed the preference for straw ( $F_{2,486}=6.67$ ;  $P<0.001$ ). HFP chickens spent more time in the straw pens compared to CON (lsmeans 46% vs. 38%,  $P<0.01$ ) and LFP (lsmeans 40%,  $P<0.05$ ). CON and LFP did not differ significantly. The lines also differed with respect to preference for peat ( $F_{2,486}=5.79$ ;  $P<0.01$ ). HPF chickens spend less time in the peat pens compared to CON (lsmeans 19% vs. 24%,  $P<0.01$ ) and LFP (lsmeans 19% vs. 25%,  $P<0.01$ ) and again CON and LFP did not differ. The lines did not differ in their relative preference for sand.

### 3.2. Experiment 2: consumer demand approach

#### 3.2.1. Total number of visits to the choice pens

One LFP hen was excluded from the experiment because she did not learn to open the push doors. Thus, analysis was performed on seven LFP hens and eight HFP hens. In total, 10 hens worked for getting access to peat moss (four LFP hens, six HFP hens), 10 hens worked for getting access to sand (four LFP hens, six HFP hens), 11 hens worked for getting access to straw (six LFP hens, five HFP hens) and seven hens worked for getting access to the wire floor (four LFP hens, three HFP hens). Figure 2 shows the frequency of visits per choice pen plotted against the door weights for both selection lines. For all choice pens, visit frequency decreased with increasing weight. At 250 g, LFP birds had a tendency for more entries to the wire pen as compared to HFP birds ( $U=12.0$ ,  $P=0.07$ ), but no other significant differences in visit frequency per weight point were found. The slopes of the demand functions for the number of entries to the choice pens for both lines are shown in Table 1. Although some slopes were steep and others were shallow, no significant differences were found between the lines. Figure 3 shows the percentage of time spent in the different choice pens per weight category for both selection lines. LFP birds spent more time in the wire pen as compared to HFP birds at the lowest weights (150 g:  $U=9.0$ ,  $P=0.10$ ; 250 g:  $U=9.0$ ,  $P=0.05$ ). The slope of the demand curve for the wire floor was significantly steeper for LFP birds as compared to HFP birds ( $t=-2.20$ ,  $P<0.05$ ; Table 1). Maximum weights pushed to enter a choice pen were low and not significantly different between both selection lines for each substrate and the wire floor (Table 2), and the same was found for HP (weight where half of the total weight is pushed, data not shown). Also no differences were found between the lines for total expenditure (Table 3). However, the relative price (RP, total price for a substrate as a percentage of the total weight pushed by an animal over all substrates) for peat moss was

significantly higher for HFP hens as compared to LFP hens ( $F_{10,1} = 6.68$ ,  $P < 0.05$ ; mean percentage HFP hens 49.2% vs. 3.3% for LFP hens).

### 3.2.2. Visits with dustbathing

No dustbathing was observed in sand, and also no sham dustbathing was observed in the wire pen. Only a small number of hens performed dustbathing behaviour. In total five hens dustbathed in peat (one LFP hen and four HFP hens), and four hens dustbathed in straw (two LFP hens and two HFP hens). Because of these small number of hens performing dustbathing behaviour we could not calculate slopes for the demand curves. The maximum weights and total expenditure for dustbathing in peat moss and straw are shown per line in Table 2 and 3. No significant differences were found. Only a tendency for a higher total expenditure for dustbathing in peat moss was found for HFP birds as compared to LFP birds (Table 3).

## 4. Discussion

Previous research showed that to prevent feather pecking, substrates that can be manipulated are preferred over substrates that can only be pecked and swallowed (Huber-Eicher *et al.*, 1997, 1998; Aerni *et al.*, 2000). An important question is if birds that differ in genetic susceptibility for feather pecking also differ in their preference for substrates, so that feather pecking can be prevented by providing them substrate best fitting to their needs. The results of the preference test carried out during rearing clearly showed that HFP pullets prefer straw as substrate whereas peat was less preferred as compared to LFP and control birds.

However, the results of the preference test could not be confirmed in the second experiment using the consumer demand approach. This may indicate that HFP hens do not defend consumption of straw to a large extent relative to LFP hens. HFP hens even seem to

consider straw as a luxury, as the slope of the demand curve was steep. However, large individual variation between the birds was found and possibly significant differences could be found with a higher number of birds in the second experiment. It is not likely that substrate preferences may differ between the rearing period and the laying period. It has been shown that substrate preferences develop during rearing and can still be found at a later age (Van Liere *et al.*, 1991; Vestergaard *et al.*, 1993; Nicol *et al.*, 2001).

In general, both LFP and HFP hens did not defend consumption of substrates to a large extent, as they both worked predominantly at lower weights. Maximum prices paid to enter a choice pen were very low as compared to ISA Brown hens in a previous study (de Jong *et al.*, 2005). Probably the push door operant task was not a very naturalistic task for hens of both selection lines which may explain why these hens were not motivated to work at higher weights. The body weights of the White Leghorn hens were much lower than that of the ISA Brown hens used in a previous experiment (mean weight 1124 g for the hens used in the present experiment vs. 1958 gr for ISA Brown hens used in a previous experiment (de Jong *et al.*, 2005)), which may also explain that the White Leghorn hens worked predominantly at lower weights.

Dustbathing behaviour was not observed in sand but only in straw and peat moss. This partially confirms the results of a previous experiment in which hens could chose between sand, peat moss and wood shavings and almost exclusively dustbathed in peat moss (de Jong *et al.*, 2005). Surprisingly, only a few hens showed dustbathing behaviour, and hens were not willing to work hard to enter a substrate to perform dustbathing behaviour. During early rearing the hens were housed on wood shavings, which may have affected their substrate preference for dustbathing behaviour. It has been shown that rearing conditions largely affect the hen's preferences at a later age (Van Liere *et al.*, 1991; Nicol *et al.*, 2001). On the other hand, hens were familiar with the other substrates (straw, peat moss, sand) which were

available in the second half of the rearing period. The low frequency of dustbathing might be a consequence of the experimental setup rather than of a low dustbathing motivation. This would also explain why the birds worked so little in general to get access to the choice pens. HFP birds tended to work harder to take a dustbath in peat moss, although these results should be interpreted with care due to the small number of animals that performed dustbathing. Surprisingly, there was a also tendency for HFP birds to work harder for peat moss as the relative price (RP) for peat moss was higher in HFP birds as compared to LFP birds, which is in contrast to the findings of the preference test. During rearing HFP pullets had a relatively lower preference for peat moss as compared to LFP pullets.

At lower weights LFP hens seemed to have a higher preference for the wire floor as compared to HFP hens, as they had a higher visit frequency and also spent more time on wire. Since the present experiment did not control for the value of the operant task itself or for that of occupying additional space or exploring the environment (Cooper, 2004), it may also be that LFP hens worked harder for the latter ‘rewards’ as compared to the HFP hens at lower weights.

## **Conclusions**

HFP birds showed a relative high preference for straw and a low preference for peat during rearing as compared to LFP and control birds. This suggests that substrate preferences may differ in birds differing in genetic susceptibility for feather pecking. However, these results could not be confirmed using a consumer demand approach during laying. This may be due to the small number of birds in the second experiment and the large individual differences between the birds. On the other hand, this may also implicate that HFP birds do not defend the consumption of straw to a large extent. More research is necessary to determine if the relative preference for straw in HFP pullets can also be found during the laying period.

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**Table 1.** The means of the slopes of the demand functions  $\pm$  SEM for each resource for both selection lines.

	total number of visits		percentage of observed time	
	LFP	HFP	LFP	HFP
peat moss	$-0.50 \pm 0.32$	$-0.65 \pm 0.12$	$-0.66 \pm 0.54$	$-0.91 \pm 0.52$
straw	$-0.89 \pm 0.18$	$-1.40 \pm 0.61$	$-1.23 \pm 0.39$	$-2.043 \pm 1.19$
sand	$-0.96 \pm 0.34$	$-0.65 \pm 0.10$	$-1.51 \pm 1.26$	$-1.082 \pm 0.68$
wire	$-1.03 \pm 0.24$	$-0.42 \pm 0.18$	$-1.24 \pm 0.74^a$	$-0.34 \pm 0.15^b$

<sup>a,b</sup>  $P < 0.05$ .

**Table 2.** Maximum price paid (g)  $\pm$  SEM to enter a particular resource, or to enter a particular resource to perform dustbathing behaviour for both selection lines.

	all entries		dustbathing	
	LFP	HFP	LFP	HFP
peat	250 $\pm$ 129	236 $\pm$ 95	25 $\pm$ 25	175 $\pm$ 94
straw	342 $\pm$ 185	286 $\pm$ 115	50 $\pm$ 32	94 $\pm$ 70
sand	167 $\pm$ 83	278 $\pm$ 165	*	*
wire	167 $\pm$ 83	100 $\pm$ 66	*	*

\* Dustbathing was not observed on sand or on the wire floor

**Table 3.** Total expenditure (kg)  $\pm$  SEM calculated for all entries to the resources or for dustbathing behaviour for both selection lines.

	all entries		dustbathing	
	LFP	HFP	LFP	HFP
peat	0.942 $\pm$ 0.703	7.040 $\pm$ 4.955	0.025 $\pm$ 0.025 <sup>a</sup>	0.530 $\pm$ 0.255 <sup>b</sup>
straw	11.033 $\pm$ 7.455	8.200 $\pm$ 4.746	0.050 $\pm$ 0.032	0.130 $\pm$ 0.110
sand	5.200 $\pm$ 3.512	2.330 $\pm$ 1.457	*	*
wire	3.691 $\pm$ 1.741	1.050 $\pm$ 0.825	*	*

<sup>a,b</sup> Tendency for a difference (P<0.10)

\* No dustbathing observed in sand or on the wire floor

## Figure captions.

Figure 1. Schematic drawing of the test pen.

Figure 2. The number of entries per weight category per line for peat moss, sand, straw and the wire floor. Different letters indicate a tendency for a difference between HFP and LFP birds ( $P=0.07$ )

Figure 3. The percentage of time spent in the choice pens per line for peat moss, sand, straw and the wire floor. <sup>a,b</sup> Indicates a tendency for a difference between HFP and LFP birds ( $P=0.10$ ), and <sup>c,d</sup> indicates a significant difference between LFP and HFP birds ( $P=0.05$ ).

Figure 4. The number of entries per weight category per line for dustbathing in peat moss and straw.

Figure 1.

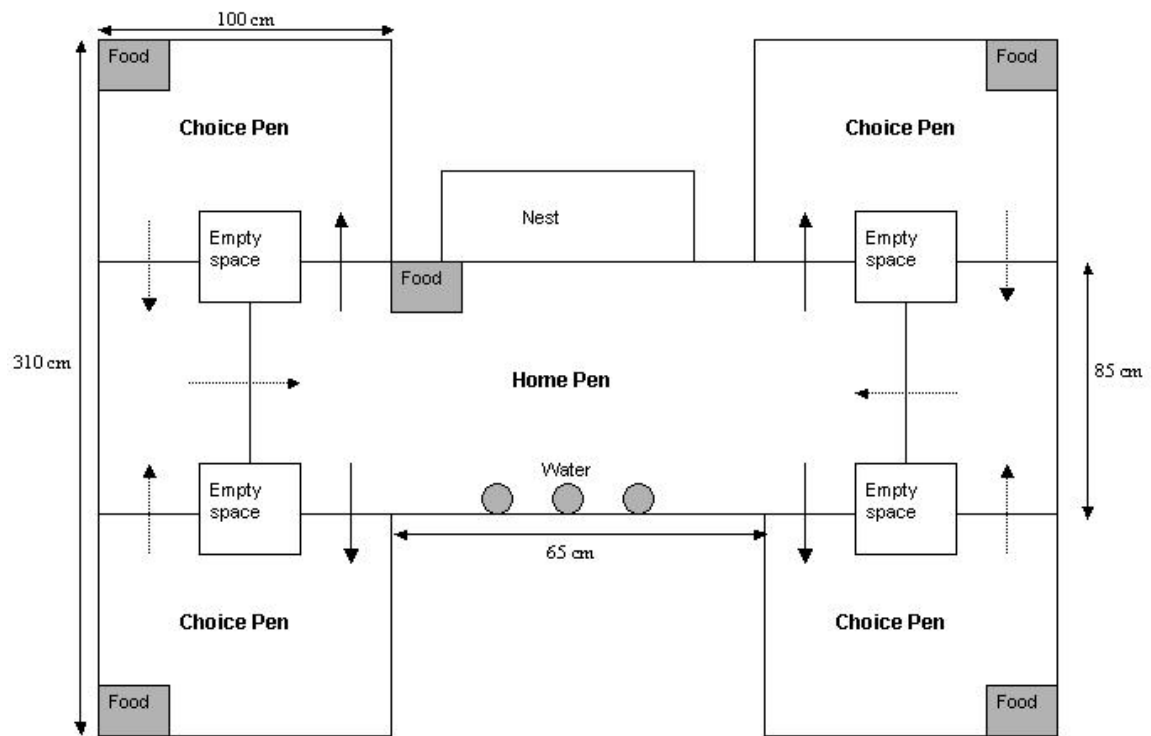


Figure 2.

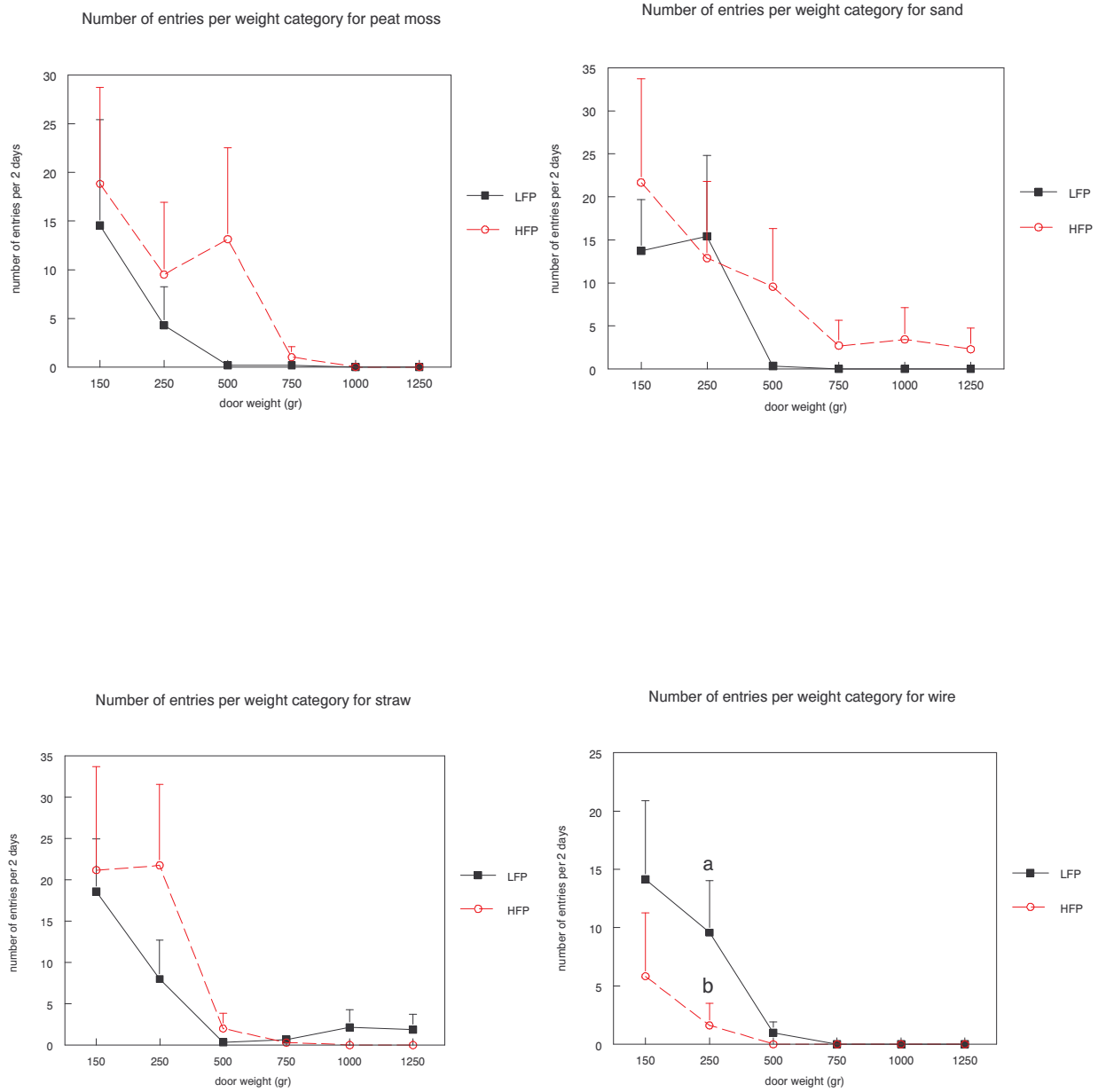




Figure 3.

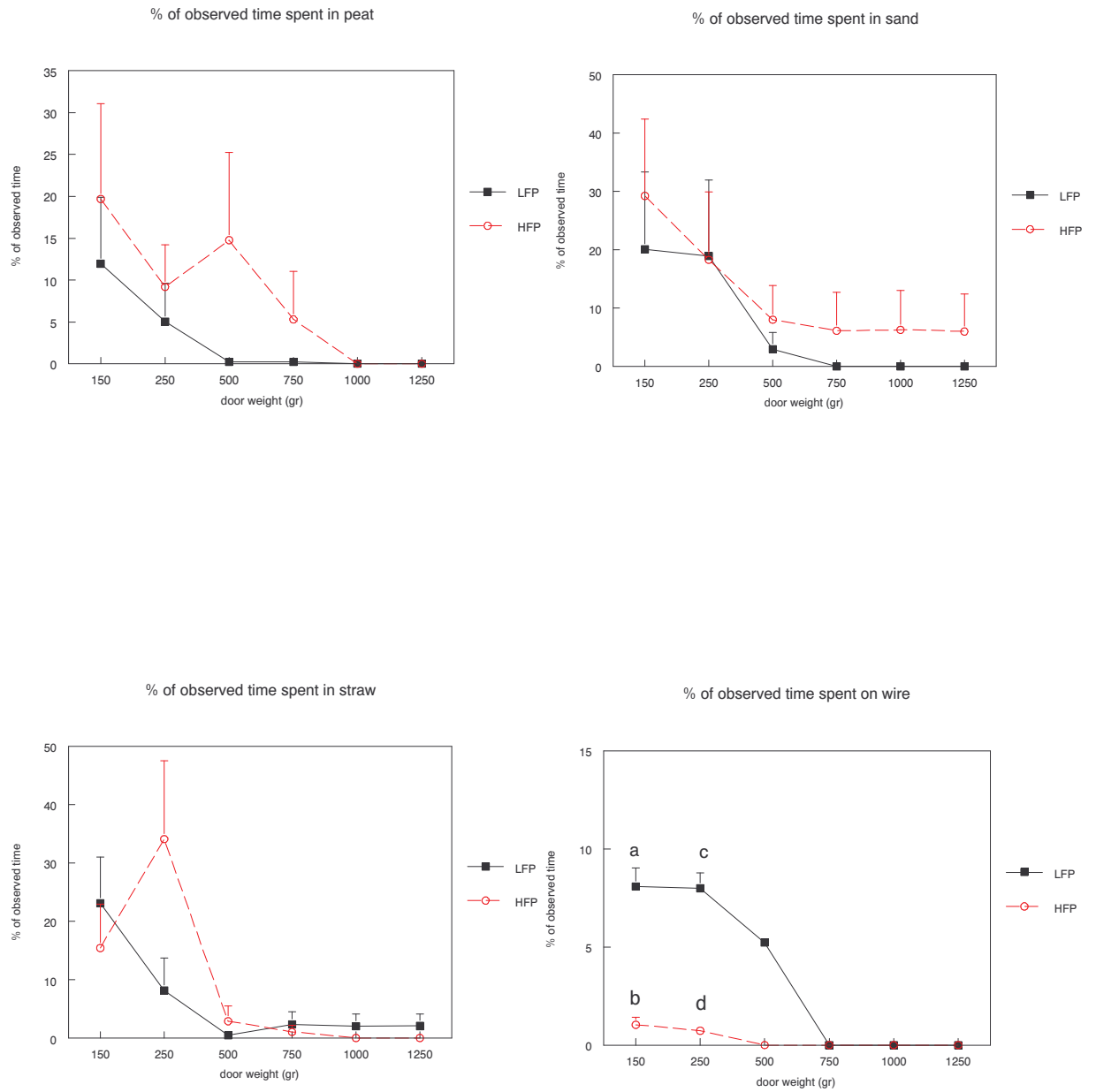


Figure 4.

