Behavior of Broiler Chickens in Four Different Substrates: a Choice Test

ABSTRACT
The aim of this study was to determine the selection of bedding material by broiler chickens during the rearing period and whether the choice was determinant to their performing a specific behavior. To achieve this objective, a choice test was designed. A choice pen was constructed where birds could move freely around the four selected materials (straw, wood shavings, rice hulls and sand). Chickens were introduced in this pen in four groups of eight birds, three days a week for one hour per day and group, for four weeks. The location and the activity performed by each broiler were recorded every five minutes. Results showed a preference for sand compared with the other three substrates. However, differences between the behaviors performed in each bedding material were shown mainly for resting (preferably performed on wood shavings and straw), dust bathing (on sand), pecking and scratching (on rice hulls). Other factors, such as the time of day, were also found to have effects on fighting and drinking, and changes in behavioral patterns (resting, preening, eating, standing and pecking) were also detected as broilers grew older.

INTRODUCTION
In poultry production, attempts have been made to use and test several types of material for litter: refined gypsum (Wyatt & Goodman, 1992; Grimes et al., 2006), recycled paper chips from waste newspapers (Lien et al., 1992), pelleted newspaper (Frame et al., 2002; Grimes et al., 2006), tree core-like kenaf core (Malone et al., 1990; Brake et al., 1993), particleboard residues (Hester et al., 1997), leaves (Willis et al., 1997), sand (Bilgili et al., 1999; Arnould et al., 2004), cotton waste (Grimes et al., 2006), hazelnut husks or wheat stalks (Sarica & Cam, 2000), wood shavings (Shields et al., 2005; Macklin et al., 2005), rice hulls (Swain & Sundaram, 2000; Shields et al., 2004), rice hull ashes (Chamblee et al., 2003), coffee husk (Ortiz et al., 2003; Ortiz et al., 2006), saw dust (Mendes et al., 2011), coir dust (Swain & Sundaram, 2000), straw (Al Homidan & Robertson, 2003), feathers (Sanotra et al., 1995; Gunnarson et al., 2000), sugarcane bagasse (García et al., 2010) or peat (Petherick & Duncan, 1989). The use of any of these substrates most often depends on the availability in each area and at each moment.
proposed as bedding for poultry (e.g. Sanotra et al., 1995; Willis et al., 1997; Al Homidan & Robertson, 2003). The addition of other products to improve bird performance and litter characteristics is also being investigated (e.g. natural zeolite in Eleroglu & Yalcin, 2005; alum in Worley et al., 1999).

Moreover, the characteristics of the materials used as broiler substrate must be taken into account, because some substrates may enrich the environment and support important behaviors of the birds (Gunnarson et al., 2005; alum in Worley et al., 1999). Investigated (e.g. natural zeolite in Eleroglu & Yalcin, 2005). The addition of other products to improve bird performance and litter characteristics is also being investigated (e.g. natural zeolite in Eleroglu & Yalcin, 2005; alum in Worley et al., 1999).

The second objective of this study was to determine the predominant behaviors on each of these materials.

**MATERIAL AND METHODS**

**Animals and housing**

In this study, 40 one-day-old male Rossä broiler chicks (Aviagen, Alabama, USA) were obtained from a commercial hatchery. Birds were housed in the same home pen and marked with rings on their right legs in groups of eight birds. Four ring colors were used, one for each bird group, and a group of eight birds had no mark, so these could be used to replace any of the marked broilers, if needed. This replacement was never necessary, so 32 chickens were finally used in the choice test experiment. The home pen measured 2x2 m and contained wood shavings, sand, rice hulls and straw. These substrates were located in four different sections of the pens, each section measuring 1 m² (see Figure 1) with a substrate depth of 15 cm. The chickens could move freely around the 4 m² pen, so they had permanent access to the four materials. In the center of the pen a drinker and a feeder were located, so that broilers could drink or eat from any part of the pen and, in consequence, while standing on any of the four substrates. Feed and water were provided ad libitum and for the first three days, chickens had 24 hours light, which was then gradually decreased to a 16L:8D schedule. No birds died during the experiment.

**Choice test**

Choice test was carried out when birds were between 14 and 42 days old (for four weeks), three days per week according to the following protocol. One of the marked groups was transferred from the home pen to the choice test pen, which was located in the same room. This pen was exactly the same as the home one, as shown in Figure 1, and the birds could freely access the four studied substrates, a feeder and a drinker.

Once the eight chickens were in the choice test pen, the observer sat quietly about 2 meters away from the front of the pen and the chicks were given 10 minutes.
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The experimental design was approved by the Ethical and Animal Welfare Committee of the Valencian Institute of Agricultural Research.

Statistical analyses

Original data were converted into frequencies, so that in each observation (every five minutes) there was only one data item including the eight animals, expressing the relative percentage of animals that were performing one activity on a specific substrate. The experimental unit was the group, with bird nested within group.

General distribution of frequencies was analyzed using a chi-squared test (procedure FREQ of SAS® System, SAS Institute 2009). Subsequently, in order to identify the activities that were preferably performed by the broiler chickens on each of the four studied substrates, a factorial discriminant analysis, using stepwise option, was run with typified variables (STEPDISC procedure from SAS System, SAS Institute, 2009).

On the other hand, in order to assess the effect of each substrate, bird age and time of day on the different observed behaviors, a logistic regression model was performed (Agresti, 1990), using procedure GENMOD of SAS System (SAS Institute, 2009), assuming a binomial distribution and using logit as the link function. In addition, the GLIMMIX procedure by SAS System (SAS Institute, 2009) produced the estimates of the average log it on the scale of the data.

The evaluated effects were substrate type, the time of day (because the test was carried out during the entire morning each day, and it was possible that the time of day had some influence) and bird age, so repeated measures were included. For this effect, it was decided to divide the test into two different ages: from the beginning (14 days) to 21 days old (the middle of the rearing period) and from day 21 to the end (day 42). The interaction between substrate and age was also evaluated. As a result, the equation (1) shows the logistic regression model:

$$L_{ijkl} = \log(\frac{P_{ijkl}}{1-P_{ijkl}}) = \beta_0 + \beta_1 \cdot T + \beta_2 \cdot A + \beta_3 \cdot S + \beta_4 \cdot A \cdot S + e_{ijkl}$$

Where: $L_{ijkl}$=linear logistic model; logit($P_{ijkl}$): the probability of a categorical response ($P_{ijkl}$=probability of “positive activity” response); $1-P_{ijkl}$=probability of “absence of activity” response; $\beta_0$=intercept; $\beta_1$, $\beta_2$, $\beta_3$ = coefficients estimated for the logistic regression models; $T$=Time of the day (from 1 to 240 minutes);

The observed behaviors were classified into the following categories: resting, preening, walking, eating, drinking, dust bathing, fighting, standing, pecking, scratching, and flapping. This ethogram is explained in Table 1.

### Table 1 – Developed ethogram during the choice test

<table>
<thead>
<tr>
<th>Activity</th>
<th>Definition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resting</td>
<td>When the bird sat or lay down on the floor, without any other activity</td>
</tr>
<tr>
<td>Preening</td>
<td>When the bird arranged its feathers with its beak</td>
</tr>
<tr>
<td>Walking</td>
<td>When the bird walked in the pen</td>
</tr>
<tr>
<td>Eating</td>
<td>When the bird ate, regardless of whether it was standing, sitting or resting</td>
</tr>
<tr>
<td>Drinking</td>
<td>When the bird drank, regardless of whether it was standing, sitting or resting</td>
</tr>
<tr>
<td>Dust bathing</td>
<td>When the bird forced the material into the plumage by squattting on the ground and making appropriate movements with the body, wings and legs</td>
</tr>
<tr>
<td>Fighting</td>
<td>When the bird was fighting against any conspecific</td>
</tr>
<tr>
<td>Standing</td>
<td>When the bird was just standing, no other activity</td>
</tr>
<tr>
<td>Pecking</td>
<td>When the bird pecked any part of the substrate</td>
</tr>
<tr>
<td>Scratching</td>
<td>When the bird moved the litter backwards with its feet</td>
</tr>
<tr>
<td>Flapping</td>
<td>When the bird opened and moved both of its wings energetically</td>
</tr>
</tbody>
</table>

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$$L_{ijkl} = \logit(P_{ijkl}) = \log\left(\frac{P_{ijkl}}{1-P_{ijkl}}\right) = \beta_0 + \beta_1 \cdot T + \beta_2 \cdot A + \beta_3 \cdot S + \beta_4 \cdot A \cdot S + e_{ijkl}$$

Where: $L_{ijkl}$=linear logistic model; logit($P_{ijkl}$): the probability of a categorical response ($P_{ijkl}$=probability of “positive activity” response); $1-P_{ijkl}$=probability of “absence of activity” response; $\beta_0$=intercept; $\beta_1$, $\beta_2$, $\beta_3$ = coefficients estimated for the logistic regression models; $T$=Time of the day (from 1 to 240 minutes);
A = effect of age, (age ≤ 21 day, considered as age 1; or age > 21 day, considered as age 2); S = effect of litter material (k = 1, straw; k = 2, wood shavings; k = 3, rice hulls; sand, used as reference); εijkl = residual error. Likelihood ratio tests were performed among nested models for computing likelihood-based fit statistics.

RESULTS

Figure 2 shows the general distribution of the frequencies of the presence of the birds on each of the four studied substrates. The frequency of the birds on sand was the highest, followed by wood shavings ($\chi^2 = 1867.91$, $p < 0.0001$).

The discriminant analysis maximizes the differences between the four floor substrates. It uses linear combinations of the frequencies of the performed activities by the birds on each bedding material. The selected functions using the “stepwise” option in factorial discriminant analysis are shown in Table 2 and graphic results of this analysis are observed in Figure 3.

In Table 2, only resting, preening, eating, drinking, dust bathing, pecking and scratching are displayed, given their statistically significance ($p < 0.05$) in the model, while walking, sitting, standing or flapping did not. This means that they do not help to explain the differences between the bedding materials.

The first discriminant function divides substrates where preening, eating and dust bathing (positive values) were preferentially performed, from those which presented opposite values, mainly resting and drinking. On the contrary, the second discriminant function differentiates the substrates where preening, drinking and dust bathing performed from those where birds demonstrated pecking and scratching.

Combining these two discriminant functions, Figure 3 was obtained, where bird groups are distinguished among substrate. This figure shows that wood shavings and straw were very similar because they clustered, while rice hulls and sand were clearly differed from both and even between each other. Therefore, three groups were clearly differentiated and it was expected that the behaviors performed in each of those groups were different.

Relative to the application of the logistic regression model, Table 3 shows the significance of each effect evaluated in the logistic model, and it may be observed that the proposed logistic model fitted all of the activities, except for flapping.

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It is also observed that depending on the behavior, the significance of the effects differs. The effect of substrate was statistically significant for all behaviors,
Figure 4 – Frequencies of each behavior influenced by age and substrate

- **Resting**
  - Feeding:
    - Age: 21 d
    - Age: 21 d
  - Drinking:
    - Age: 21 d
  - Age: 21 d

- **Dust bathing**
  - Age: 21 d
  - Age: 21 d

- **Preening**
  - Age: 21 d
  - Age: 21 d

- **Standing**
  - Age: 21 d
  - Age: 21 d

- **Walking**
  - Age: 21 d
  - Age: 21 d

- **Pecking**
  - Age: 21 d
  - Age: 21 d

- **Eating**
  - Age: 21 d
  - Age: 21 d

- **Scratching**
  - Age: 21 d
  - Age: 21 d

\[\text{Means within a graph with no common superscript differ significantly (p<0.05)}\]
except for fighting and standing, whereas the effects of the time, bird age and their interaction with substrate effects were more dependent on each behavior.

Once the logistic regression model was fitted, coefficients were obtained for each behavior (Table 4). These coefficients reveal the real influence of time, age and substrate on each behavior; the higher the value of the $\beta$ coefficient, the greater the frequency of that behavior in relation to sand and age >21 days, which were used as references in the logistic model. Similarly, the lower the $\beta$ coefficient, the lower the frequency of a behavior. For instance, compared with sand, scratching was performed more in any other substrate, although the highest frequency took place on rice hulls, as $\beta$ was the highest estimated logistic regression coefficient.

### Table 4 – Estimated coefficients throughout the Logistic Regression Model for the different behaviors

<table>
<thead>
<tr>
<th>Behavior</th>
<th>$\beta_0$</th>
<th>$\beta_1$ (time)</th>
<th>$\beta_2$ (age1)</th>
<th>$\beta_3$ (straw)</th>
<th>$\beta_3$ (wood shavings)</th>
<th>$\beta_3$ (rice hulls)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Resting</td>
<td>-1.762</td>
<td>-0.741</td>
<td>-0.167</td>
<td>-0.199</td>
<td>-1.277</td>
<td></td>
</tr>
<tr>
<td>Preening</td>
<td>-3.025</td>
<td>-1.371</td>
<td>-1.783</td>
<td>-1.188</td>
<td>-1.889</td>
<td></td>
</tr>
<tr>
<td>Walking</td>
<td>-4.400</td>
<td>0.001</td>
<td>-1.393</td>
<td>-0.290</td>
<td>-1.210</td>
<td></td>
</tr>
<tr>
<td>Eating</td>
<td>-3.072</td>
<td>0.503</td>
<td>-3.587</td>
<td>-2.485</td>
<td>-1.667</td>
<td></td>
</tr>
<tr>
<td>Drinking</td>
<td>-7.571</td>
<td>0.020</td>
<td>0.697</td>
<td>2.787</td>
<td>2.9615</td>
<td>0</td>
</tr>
<tr>
<td>Dust-bathing</td>
<td>-2.301</td>
<td>-1.200</td>
<td>-27.185</td>
<td>-3.456</td>
<td>-27.185</td>
<td></td>
</tr>
<tr>
<td>Fighting</td>
<td>-5.560</td>
<td>-0.087</td>
<td>0.693</td>
<td>0</td>
<td>1.102</td>
<td>0</td>
</tr>
<tr>
<td>Standing</td>
<td>-4.892</td>
<td>-0.984</td>
<td>-0.985</td>
<td>-0.473</td>
<td>-0.697</td>
<td></td>
</tr>
<tr>
<td>Pecking</td>
<td>-2.948</td>
<td>-1.015</td>
<td>-1.191</td>
<td>-0.964</td>
<td>0.697</td>
<td></td>
</tr>
<tr>
<td>Scratching</td>
<td>-6.296</td>
<td>0.289</td>
<td>1.101</td>
<td>0.983</td>
<td>3.803</td>
<td></td>
</tr>
<tr>
<td>Flapping</td>
<td>-7.781</td>
<td>-25.324</td>
<td>-25.327</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

All the values shown in Table 4 and those related to the interaction between age and substrate were used to construct Figure 4 and Figure 5 (drinking and fighting). These figures illustrate more clearly than Table 4 that certain behaviors were preferably performed on some substrates. For example, dust bathing was mainly performed on sand, and pecking and scratching were mostly developed on rice hulls. Likewise, the effect of age on each behavior and substrate is clearly shown.

The most remarkable results were obtained for resting, dust bathing, pecking, scratching and preening. Resting was performed mainly on wood shavings and straw, which were preferred over sand and rice hulls (although when birds were older, they also tended to rest on sand, as shown in Figure 4). On the other hand, age tended to decrease the probability of this behavior, although an interaction with substrate was present, so this pattern changed to sand. In contrast, sand was strongly preferred for dust bathing, while rice hulls were chosen for pecking and scratching. In addition, preening was performed in all substrates, although wood shavings and sand were those in which preening was most frequent. Age also had a notable effect on this behavior and it was coincident with dust bathing tendency with age, and occurred mainly on wood shavings and sand.

**DISCUSSION**

Sand was strongly selected over the other three types of litter in terms of the general results, but this pattern changed when individual behaviors were assessed. Although the current substrate can determine...
the behavior or the chosen substrate in laying hens (Nicolet et al., 2001), that was not possible in our study because broilers were able to access the four bedding materials in the home pen, so in this study sand was clearly chosen.

When behaviors were separately evaluated, sand was mainly chosen for dust bathing. This preference for sand was previously reported by Shields et al. (2004) and Sanotra et al. (1995), and it is quite clear that if the birds have the possibility, they perform these baths on sand. In addition, it was observed that wood shavings were more frequently chosen than rice hulls or straw to perform dust bathing. Simultaneously, preening was mainly performed on sand when birds were older than 21 days and on wood shavings when they were younger than 21 days. These results may indicate that it is a kind of behavior following or prior to the bath, as it coincides with dust bathing results, although the effect of the substrate itself is less clear.

Broilers selected rice hulls for pecking and scratching, so they did not peck more frequently on the same substrate they preferred for dust bathing, as suggested by Shields et al. (2005) and Arnould et al. (2004). Therefore, our results are not consistent with those that suggest that the behaviors of pecking and scratching are previous to dust bathing. However, pecking frequency may be related to dust bathing, because it increased when birds were older than 21 days, which coincided with the higher frequency of dust bathing. However, a possible explanation for our findings is that pecking and scratching are exploratory behaviors, and therefore certain substrates that are friable, such as rice hulls, may attract the birds to peck or scratch. On the other hand, this material may not be adequate or stimulating for dust bathing because it maybe too abrasive. The present results cannot be compared with other studies, such as that of Sanotra et al. (1995) because they did not use rice hulls as substrate; however, they found that scratching was very frequent in straw, and that scratching and pecking preferences were different from those for dust bathing. Gunnarsson et al. (2000) also reported that straw was preferred for pecking and scratching, but not for dust bathing, but did not test rice hulls either.

The hypothesis that rice hulls may be abrasive is supported by there sting frequency results. Broilers selected to rest on, in decreasing order wood shavings, straw, sand, and rice hulls, indicating that wood shavings and straw are more comfortable than rice hulls. Furthermore, it must be remembered the strong tendency of chickens to peck and scratch on rice hulls, and therefore, other behaviors may be much less likely to occur. Relative to resting on wood shavings and sand, our results were opposite to the findings of Shields et al. (2005), who observed that resting as well as active behaviors were performed more on sand; however, they did not test sand as substrate. Lindner et al. (1997) also observed that resting frequency was significantly higher on wood chips than on straw.

Walking and standing were studied as they indicated general activity, but as seen in Figure 4, their frequencies were very low (maximum of 1.2% and 0.8%, respectively), and therefore, no conclusions can be drawn from these data.

Taking all these findings into account, more than one litter substrate should be available to broilers on the farms to allow them to perform some behaviors, which may be compromised in current conditions.

Age influenced some behaviors (Table 3), differently from the findings of Shields et al. (2004). The individual frequency of the evaluated behaviors decreased with age (β, in Table 4), except eating, which increased as birds aged. However, as shown in Figure 4, the interaction between age and substrate had a different effect, and the frequency of certain active behaviors (such as dust bathing, pecking and scratching), tended to increase with age when birds were on determined substrates, which is just the opposite to that found by Shields et al. (2005). A possible hypothesis to explain our results is the design of the choice test. Broilers were transferred to the choice test pen every experimental day, but they lived in the home pen, which litter became gradually dirty. On the other hand, bedding materials in the choice pen they were clean, unused and attractive, and therefore the birds preferred to explore and use them instead of eating or resting on them. This exploratory behavior may also explain the lack of observations in which the birds were just walking or standing, as mentioned above.

On the other hand, the effect of time of day was statistically significant on certain behaviors (Table 3). Fighting decreased as the morning advanced. Although we hypothesized that this may be due to general activity reduction over the morning, the remaining active behaviors did not confirm this hypothesis, and we did not find any literature regarding the daily pattern of these behaviors. The strong increase in drinking behavior shown in Figure 5 during the morning maybe due to increase in room temperature, although these data were not available and therefore further research is necessary to identify the causes of these findings.
CONCLUSIONS

According to our findings, broilers prefer sand to any other bedding material, despite the observed behavioral differences. Broilers tend to dust bathe when sand is available, and they do not tend to perform this behavior on other substrates. On the other hand, rice hulls are quite recommendable to provide a litter where birds can perform exercise behaviors, such as scratching or pecking, while wood shavings or straw seem to be considerably less attractive in general, except for inactive behaviors. Consequently, there are apparent differences in the behaviors performed on each bedding material, as well as a complex relationship between them.

REFERENCES
Kirkden RD, Pajor EA. Using preference, motivation and aversion tests to ask scientific questions about animals’ feelings. Applied Animal Behavior Science 2006;100:29-47.


