Revista Brasileira de Ciência Avícola
ISSN: 1516-635X
revista@facta.org.br
Fundação APINCO de Ciência e Tecnologia Avícolas
Brasil

Villagrá, A; Olivas, I; Althaus, RL; Gómez, EA; Lainez, M; Torres, AG
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Revista Brasileira de Ciência Avícola, vol. 16, núm. 1, enero-marzo, 2014, pp. 67-75
Fundação APINCO de Ciência e Tecnologia Avícolas
Campinas, SP, Brasil

Brazilian Journal of Poultry Science
Revista Brasileira de Ciência Avícola
ISSN 1516-635X Jan - Mar 2014 / v.16 / n.1 / 67-76

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

■Author(s)

Villagrá A[I]
Olivas I[I]
Althaus RL[II]
Gómez EA[I]
Lainez M[I]
Torres AG[III]

[I] Centro de Tecnología Animal - IVIA. Polígono La Esperanza nº100, 12400, Segorbe, Castellón, Spain
[II] Cátedra de Biofísica, Departamento de Ciencias Básicas, Facultad de Ciencias Veterinarias, Universidad Nacional del Litoral, R. P. L. Kreder 2805 (3080) Esperanza, Santa Fe, Argentina
[III] Instituto de Ciencia y Tecnología Animal, Universidad Politécnica de Valencia, Camino de Vera s/n, 46022, Valencia, Spain

■Mail Adress

Corresponding author e-mail address
Arantxa Villagrá.
Centro de Tecnología Animal - IVIA. Polígono de La Esperanza nº 100.12400, Segorbe, Castellón, Spain.
Phone: 0034. 964.71.21.15
Fax: 0034.964.71.02.18
E-mail: villagra_ara@gva.es

■Keywords

Behavior, broiler, choice test, litter, preference.

Submitted: June/2012
Approved: October/2013

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), pelletized 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.

In recent years, materials like sawdust or wood shavings have become scarce, due to the fact that they are used as fuel, so the broiler industry has had to look for new and readily available materials. In the search for new materials for broiler litter, researchers must seek materials that meet hygienic requirements and guarantee that ammonia levels do not exceed certain levels throughout the productive cycle (Worley *et al.,* 1999). In this sense, distinct mixtures of different materials have been

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.,* 2000), as well as determine chickens' skin condition (Mendes *et al.,* 2011). Thus, providing a good litter would be an effective way to increase broiler activity (Shields *et al.,* 2005) and to reduce locomotion problems (Almeida Paz *et al.,* 2010). A study carried out by Newberry (1999) assessed the effect of increasing the environment complexity on the use of broiler pen space, determining that the presence of specific materials stimulates exploratory behavior, with the consequent beneficial effects on the locomotive system of these birds. One type of exercise in birds is dust bathing, but there are other behaviors, such as pecking and scratching which are also said to be beneficial. In addition, this environmental enrichment can be devised in several ways; for example Arnould *et al.* (2004) assessed the effect of enriching experimental pens with sand trays, finding that they were attractive for chickens.

Laying hens have shown a strong preference for certain substrates such as sand to practice those cited activities (Nicol*et al.,* 2001), while broilers clearly prefer this material to perform most of their dust baths when four different substrates are made available (Shields *et al.,* 2004). In addition, it seems that the availability of sand enhances chickens' foraging activities (Arnould *et al.,* 2004). Most of these studies have focused on certain behaviors such as dust bathing, pecking or scratching, but only a few of them studied other behaviors on these substrates (e.g. Lindner & Hoy, 1997; Shields *et al.,* 2005).

Several of the cited studies have been carried out through preference or choice tests (Shields *et al.,* 2004; Sanotra *et al.,*1995). These two terms are not the same, according to Kirkden and Pajor (2006): preference denotes a difference between the strength of motivation to obtain or avoid something, while choice describes characteristics of an animal (e.g., an animal prefers bananas to oranges). In recent years, both of these tests have been used with poultry to develop preference studies with very different objectives, such as studying color in pecking devices (Jones & Carmichael, 1998), light sources (Moinard & Sherwin, 1999; Kristensen *et al.,* 2007), type of cages (Elston *et al.,* 2000) or ammonia concentrations (Kristensen & Wathes, 2000). Therefore, the first aim of the present research was to assess the selection by broiler chickens of different types of materials widely used as litter in the poultry industry (sand, wood shavings, straw and rice hulls) using a choice test. 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 Rossâ 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 m^2 (see Figure 1) with a substrate depth of 15 cm. The chickens could move freely around the 4 m^2 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

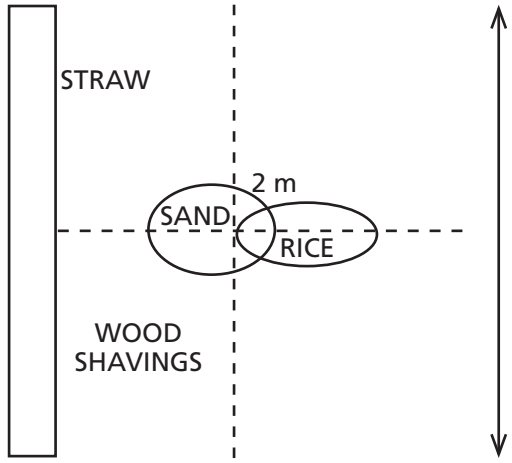


Figure 1 – Distribution of the home pen and the identical test pen

to habituate to the observer's presence and to the new pen. Then, behavioral and choice observations started. This protocol was adapted from Shields *et al.* (2005).

Every five minutes for one hour (12 observations per hour), a scan sampling was carried out, and the position of each bird and the performed behavior was recorded. After the first hour, the group was removed, the test pen was cleaned and another group was transferred from the home pen to the choice test one, and the same protocol was followed. The procedure was identical for the four groups each day the choice test was done. A Latin square was used to determine the order in which the groups had to be transferred to the choice test pen.

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

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

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 ona 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 log it as the link function. In addition, the GLIMMIX procedure by SAS System (SAS Institute, 2009) produced the estimates of the average log its 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:

$$L_{ijkl} = \log it(P_{ijk}) = \log\left(\frac{P_p}{1-P_p}\right) = \beta_0 + \beta_1 \cdot T_i + \beta_2 \cdot A_j + \beta_{3k} \cdot S_k + \beta_{4jk} \cdot A \cdot S_{jk} + \varepsilon_{ijkl}$$

Where: L_{ijkl}=linear logistic model; logit(P_{ijk}): the probability of a categorical response (P_p=probability of "positive activity" response; 1-P_p=probability of "absence of activity" response); β_0=intercept; β_1, β_2, β_3 = coefficients estimated for the logistic regression models; T_i=Time of the day (from 1 to 240 minutes);

A_j=effect of age, (age ≤21day, considered as age1; or age>21day, considered as age 2); S_k=effect of litter material (k=1, straw; k=2, wood shavings; k=3, rice hulls; sand, used as reference); ε_{ijkl} = 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 (χ^2= 1867.91, p<0.0001).

Figure 2 – Distribution of frequencies at which birds chose each studied substrate

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.

Table 2 – Coefficients of Factorial Discriminant Analysis for litter variable

Behavior	Function 1	Function 2	Function 3
Resting	-0.599	-0.342	0.429
Preening	0.473	-0.575	-1.285
Eating	0.443	0.074	0.397
Drinking	-0.800	-0.500	0.528
Dust Bathing	0.960	-0.658	0.407
Pecking	-0100	0.683	0.245
Scratching	-0.201	0.966	0.227

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.

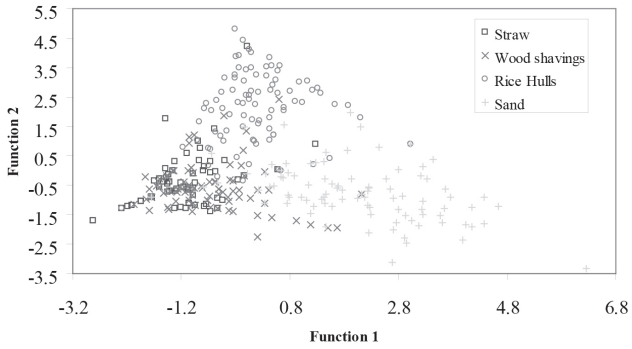


Figure 3 – Scattered plot per substrate of the relative frequencies of activities in two discriminant dimensions

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.

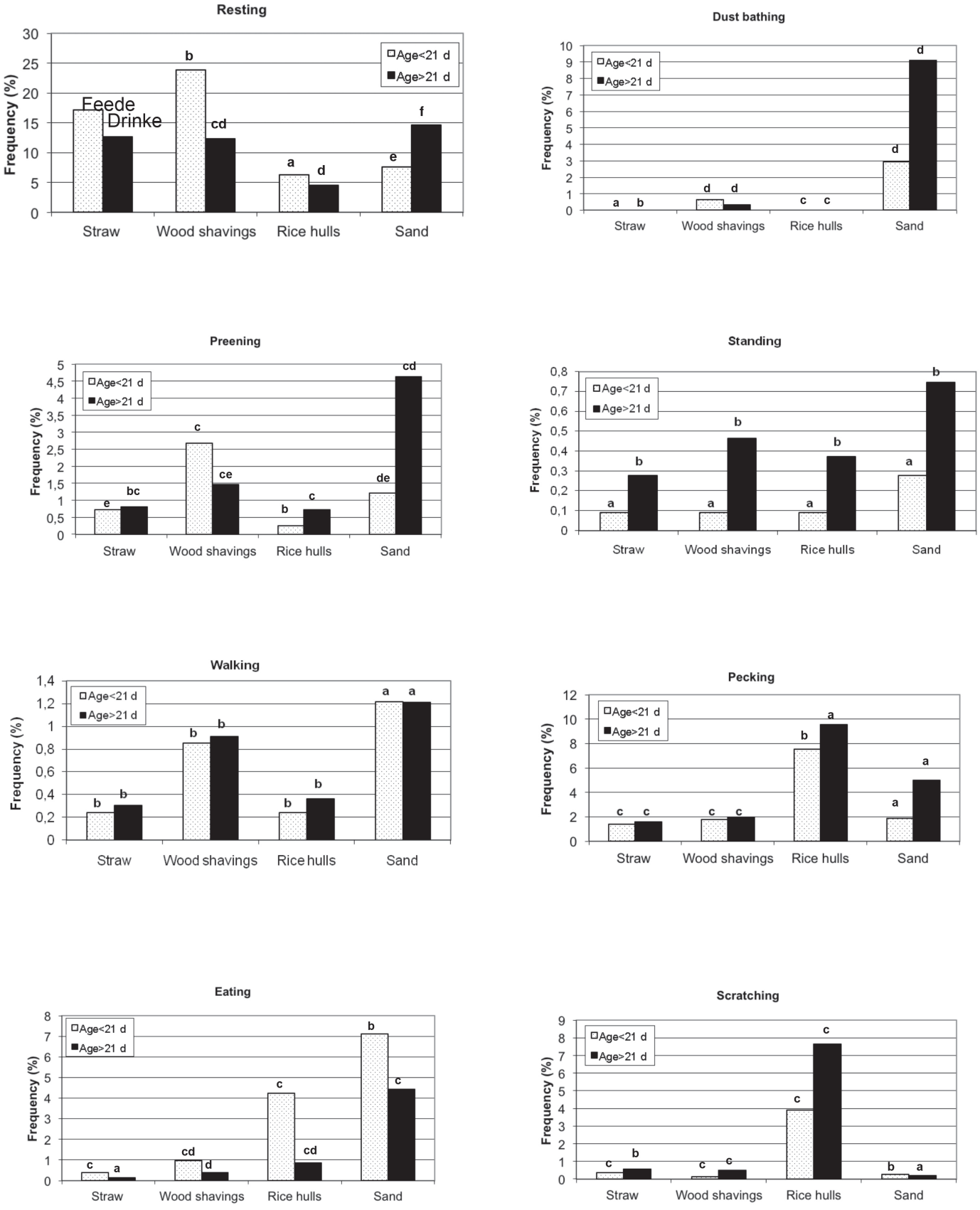
Relative to the application of the logistic regression model, Table 3 shows the significance of each effect evaluated in the logistic model, and it may be observed that the proposed logistic model fitted all of the activities, except for flapping.

Table 3 – Results of the application of Logistic Regression Model to the assessment of the effect of substrate, age and time of day on different behaviors of broiler chickens

	Effects (p-value)			
Behavior	Time	Age	Substrate	Substrate*Age
Resting	0.7622	0.0018	<0.0001	<0.0001
Preening	0.5534	0.0185	<0.0001	<0.0001
Walking	0.1021	0.5963	0.0006	0.9725
Eating	0.230	<0.0001	<0.0001	0.0005
Drinking	0.0065	0.5874	<0.0001	0.5257
Dust-bathing	0.3904	0.4991	<0.0001	0.0004
Fighting	0.0015	0.9698	1.0000	1.0000
Standing	0.7792	0.0091	0.2252	0.9582
Pecking	0.1168	0.0035	<0.0001	0.0367
Scratching	0.062	0.1559	<0.0001	0.6229
Flapping	0.3424	0.98235	1.0000	1.0000

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



[a,b,c] 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 β 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 β 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 β was the highest estimated logistic regression coefficient.

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

Behavior	β_0	β_1 (time)	β_2 (age1)	β_3 (straw)	β_3(wood shavings)	β_3 (rice hulls)
Resting	-1.762	-	-0.741	-0.167	-0.199	-1.277
Preening	-3.025	-	-1.371	-1.783	-1.188	-1.889
Walking	-4.400	-	0.001	-1.3933	-0.290	-1.210
Eating	-3.072	-	0.503	-3.587	-2.485	-1.667
Drinking	-7.571	0.020	0.697	2.787	2.9615	0
Dust-bathing	-2.301	-	-1.200	-27.185	-3.456	-27.185
Fighting	-5.560	-0.087	0.693	0	1.102	0
Standing	-4.892	-	-0.984	-0.985	-0.473	-0.697
Pecking	-2.948	-	-1.015	-1.191	-0.964	0.697
Scratching	-6.296	-	0.289	1.101	0.983	3.803
Flapping	-7.781	-	-25.324	-25.327	0	0

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.

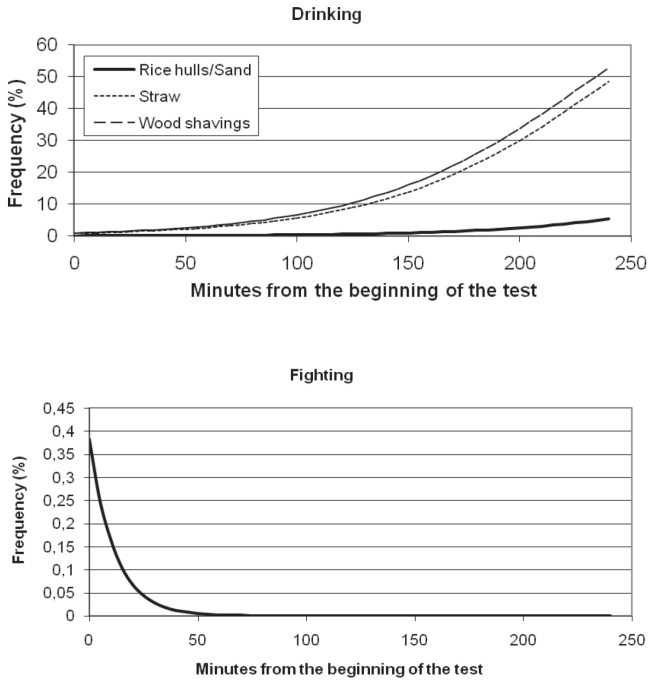


Figure 5 – Frequencies of drinking and fighting, which are influenced by time of day

Furthermore, the effect of, time of the day on certain behaviors was statistically significant, so as the morning (and hence the test) progressed, results varied. This is shown in Figure 5, where it can be observed that the tendency to fight decreased over the morning. Fighting was *a priori* of little importance, but we detected that it was only observed in the first moments of the morning and then the probability of the display of this behavior decreased (β_1=-0.087). In contrast, drinking followed a very different pattern, increasing as the morning advanced. In both cases, this pattern was found regardless of the age of the birds and the substrate on which the behavior was performed.

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 (Nicol*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_3 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

Agresti A. Categorical data analysis. New York: Wiley Ed; 1990.

Al Homidan A, Robertson JF. Effect of litter type and stocking density on ammonia, dust concentrations and broiler performance. British Poultry Science 2003;44:S7-S8.

Almeida Paz ICL, García RG, Bernardi R, Näas IA, Caldara FR, Freitas LW, Seno LO, Ferreira VMOS, Pereira DF, Cavichiolo F. Selecting appropriate bedding to reduce locomotion problems in broilers. Brazilian Journal of Poultry Science 2010;12(3):189-195.

Arnould C, Bizeray D, Faure JM, Leterrier C. Effects of the addition of sand and string to pens on use of space, activity, tarsal angulations and bone composition in broiler chickens. Animal Welfare 2004;13:87-94.

Bilgili SF, Montenegro GI, Hess JB, Eckman MK. Sand as litter for rearing broiler chickens. Journal of Applied Poultry Research 1999;8:345-351.

Brake JD, Fuller MJ, Boyle CR, Link DE, Peebles ED, Latour MA. Evaluations of whole chopped kenaf and kenaf core used as a broiler litter material. Poultry Science 1993;72:2079-2083.

Chamblee TN, Yeatman JB. Evaluation of rice hull ash as broiler litter. Journal of Applied Poultry Research 2003;12:424-427.

Eleroglu H, Yalcin H. Use of natural zeolite-supplemented litter increased broiler production. South African Journal of Animal Science 2005;35:90-97.

Elston JJ, Beck M, Alodan MA, Vega-Murillo V. Laying hen behavior 2. Cage type preference and heterophil to lymphocyte ratios. Poultry Science 2000;79:477-482.

Frame DD, Buckner RE, Anderson GL. Pelletized newspaper bedding for turkeys and its effect on brooding performance. Journal of Applied Poultry Research 2002;11:229-232.

García RG, Almeida Paz ICL, Caldara FR, Näas IA, Pereira DF, Freitas LW, Schwingel AW, Lima NDS, Graciano JD. Effect of the litter material on drinking water quality in broiler production. Brazilian Journal of Poultry Science 2010;12(3):165-169.

Grimes JL, Carter TA, Godwin JL. Use of a litter material made from cotton waste, gypsum, and old newsprint for rearing broiler chickens. Poultry Science 2006;85:563-568.

Gunnarsson S, Matthews LR, Foster TM, Temple W. The demand for straw and feathers as litter substrates by laying hens. Applied Animal Behavior Science 2000;65:321-330.

Hester PY, Cassens DL, Bryan TA. The applicability of particleboard residue as a litter material for male turkeys. Poultry Science 1997;76:248-255.

Jones RB, Carmichael NL. Pecking at string by individually caged, adult laying hens: color preferences and their stability. Applied Animal Behavior Science 1998;60:11-23.

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.

Kristensen HH, Wathes CM. Ammonia and poultry welfare: a review. World Poultry Science 2000; 56:235-245.

Kristensen HH, Prescott NB, Perry GC, Ladewig J, Ersboll AK, Overvad KC, Wathes CM. The behavior of broiler chickens in different light sources and illuminances. Applied Animal Behavior Science 2007;103:75-89.

Lien RJ, Conner DE, Bilgili SF. The use of recycled paper chips as litter material for rearing broiler chickens. Poultry Science 1992;71:81-87.

Lindner R, Hoy S. Results of ethological investigations in broiler chickens kept on straw or on deep litter from wood chips. Archiv für Geflüegelkunde 1997;61:49-52.

Macklin KS, Hess JB, Bilgili SF, Norton RA. Bacterial levels of pine shavings and sand used as poultry litter. Journal of Applied Poultry Research 2005;14:238-245.

Malone GW, Tilmon HD, Taylor RW. Evaluation of kenaf core for broiler litter. Poultry Science 1990; 69:2064-2067.

Mendes AS, Paixao SJ, Restelatto R, Reffatti R, Possenti JC, de Moura DJ, Morello GMZ, Carvalho TMR. Effects of initial body weight and litter material on broiler production. Brazilian Journal of Poultry Science 2011;13(3):165-170.

Moinard C, Sherwin CM. Turkeys prefer fluorescent light with supplementary ultraviolet radiation. Applied Animal Behavior Science 1999;64:261-267.

Newberry RC. Exploratory behavior of young domestic fowl. Applied Animal Behavior Science 1999;63:311-321.

Nicol CJ, Lindberg AC, Phillips AJ, Pope SJ, Wilkins LJ, Green LE. Influence of prior exposure to wood shavings on feather pecking, dustbathing and foraging in adult laying hens. Applied Animal Behavior Science 2001;73:141-155.

Ortiz A, Valdivie M, Elias A. Coffee husk as poultry bedding. First rearing. Cuban Journal of Agricultural Science 2003;37:21-25.

Ortiz A, Elias A, Valdivia M, Gonzalez R. Poultry litter, a way of increasing the nutritive value of highly fibrous materials. Cuban Journal of Agricultural Science 2006;40:55-60.

Petherick JC, Duncan IJH. Behavior of young domestic fowl directed towards different substrates. British Poultry Science 1989; 30:229-238.

Sanotra GS, Vestergaard KS, Agger JF, Lawson LG. The relative preferences for feathers, straw, wood shavings and sand for dustbathing, pecking and scratching in domestic chicks. Applied Animal Behavior Science 1995;43:263-277.

Sarica M, Cam MA. Potential of hazelnut husks as a broiler litter material. British Poultry Science 2000; 41: 541-543.

SAS® Institute. SAS user's guide: statistics version 9.0. Cary; 2009.

Shields SJ, Garner JP, Mench JA. Dustbathing by broiler chickens: a comparison of preference for four different substrates. Applied Animal Behavior Science 2004;87:69-82.

Shields SJ, Garner JP, Mench JA. Effect of sand and wood-shavings bedding on the behavior of broiler chickens. Poultry Science 2005;84:1816-1824.

Swain BK, Sundaram RNS. Effect of different types of litter material for rearing broilers. British Poultry Science 2000; 41:261-262.

Willis WL, Murray C, Talbott C. Evaluation of leaves as a litter material. Poultry Science 1997;76: 1138-1140.

Worley JW, Risse LM, Cabrera ML, Nolan MP. Bedding for broiler chickens: two alternative systems. Applied Engineering in Agriculture 1999;15:687-693.

Wyatt CL, Goodman TN. The utilization of recycled sheetrock (refined gypsum) as a litter material for broiler houses. Poultry Science 1992;71:1572-1576.