Litter lipid content affects dustbathing behavior in laying hens

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ABSTRACT Within the European Union, the provision of dustbathing material in layer housing systems will be compulsory beginning in 2012. In cage systems, food particles are mainly used as litter material and are provided on scratching mats by an automatic transporting system. However, because dustbathing is a means for hens to remove stale lipids from their plumage, lipid content of a substrate may be an important asset with regard to its adequacy. This study analyzes dustbathing behavior as affected by lipid content of feed used as litter material. A total of 72 laying hens of 2 genotypes (Lohmann Selected Leghorn, Lohmann Brown) were kept in 12 compartments (6 hens each). Compartments were equipped with a plastic grid floor (G) and additionally contained 3 different dustbathing trays (each 1,000 cm^2/hen) holding low-lipid (0.82%; L), normal-lipid (4.2%; N), and high-lipid (15.7%; H) food particles. The experiment began at 20 wk of life, and video recordings were done at wk 23, 26, and 29. Number of dustbaths, time spent dustbathing, average dustbath duration, foraging, and single behaviors within dustbaths were analyzed during the light period over 2 d in each observation week. Dustbaths occurred most frequently in the L compared with the N, H, and G treatments (all $P < 0.001$). Total time spent dustbathing was longest in the L treatment compared with the N and H treatments ($P < 0.001$). No difference in the average duration of single dustbaths was found between the L, N, and H treatments. However, when dustbath interruptions (less than 10 min) were excluded, the duration of single dustbaths was longer in the H compared with the L ($P = 0.009$) and N ($P = 0.024$) treatments. Foraging was most frequently observed in the N compared with the L, H, and G treatments (all $P < 0.001$). More body wing shakes occurred in the L compared with the N treatment, and the number of vertical wing shakes was higher in the N compared with the H treatment (all $P \leq 0.05$). Our results showed that preference for a dustbathing substrate increased with decreasing lipid content, implying that food particles may not be a suitable dustbathing substrate.

Key words: behavioral preference, dustbathing, foraging, litter lipid content, welfare

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INTRODUCTION

Dustbathing is recognized as an important behavioral need in laying hens and has been brought into focus by legal policies on housing system requirements for layers. Within the European Union, a dustbathing substrate in all housing systems for laying hens will be compulsory beginning in 2012, to allow hens the ability to "satisfy their ethological needs" and to enable "pecking and scratching" (European Union, 1999). Hens are highly motivated to dustbathe. Olsson et al. (2002) found that when layers were denied access to litter substrate, it triggered sham dustbathing; however, this did not satisfy their motivation to dustbathe. The purpose of dustbathing has been examined in a variety of studies. The removal of stale lipids from the plumage of hens seems to be a major function. van Liere and Bokma (1987) found that the amount of lipids on the back feathers of layers increased if dustbathing was prevented and that the original level of lipids was restored after hens were given access to litter material. In addition, dustbaths (**DB**) lasted longer when stale lipids were artificially distributed on the breast feathers of layers, as indicated by the increased numbers of side-lying and side-rubbing bouts, which have a crucial function in removing lipids (van Liere et al., 1991). In addition to regulating the amount of feather lipids and being functionally significant for feather maintenance (van Liere and Bokma, 1987), dustbathing also possibly serves to remove skin parasites (van Liere, 1992a). In practice, hens in enriched cage-housing systems are predominantly offered food particles as the litter substrate, which are automatically supplied in small

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amounts on scratching mats a few times per day. In a preceding study (Scholz et al., 2010), we found that hens do not prefer to dustbathe on food particles when alternative substrates are provided and that the lipid content of a litter substrate may play an important role in the preference of layers for a particular dustbathing material. So far, the effect of substrate lipid content on dustbathing behavior does not seem to have been investigated. Therefore, the aim of our study was to compare the number, duration, and organization of DB by laying hens that were simultaneously offered food particles as litter substrates having low- (**L**), normal- (**N**), or high-lipid (**H**) contents. We hypothesized that hens would prefer the L food particle trays for dustbathing because of an expected superior removal of stale lipids from their plumage, compared with N and H food particles. In addition, we hypothesized that the average duration of a single DB would be shorter in L food particles because of quicker removal of stale lipids from the plumage.

MATERIALS AND METHODS

Experimental Design

A total of 72 laying hens [36 Lohmann Selected Leghorn (**LSL**) and 36 Lohmann Brown (**LB**)] were housed in an experimental stable. Hens were kept in 12 compartments, which were located in a parallel position to each other. Layers were cage reared without access to litter substrate and were transferred to the experimental house at the age of 20 wk.

Within each compartment, hens were kept in groups of 6 randomly selected layers of the same genotype, and genotypes were arranged in alternate order. Compartments (floor space: 2 × 3 m, height: 2 m) provided 1 m^2 space/hen. They contained a plastic grid floor, a round feed trough, a water dispenser with drinking nipples, a perch (100 cm), and a nest box with straw filling. Hens were offered food (11.4 MJ of ME, 4.20% crude lipid, 16.2% CP, 3.85% Ca, 0.52% P) and water ad libitum. The light period was set from 0530 to 1700 h (observation in wk 21) and 0400 to 1700 h (observations in wk 24 and 27).

In each compartment, 3 litter trays were positioned parallel to each other (750 × 800 mm each) and were separated by sloped, solid side walls (620 to 1,227 mm height), which prevented hens from sitting on the partitions. Within each compartment, litter trays were supplied, in a random order, with 1 of 3 variants of food particles differing in crude lipid content. Substrates were 1) L (0.82% crude lipid), 2) N (food particles conforming to a normal layer diet and identical to the food provided at the feed troughs; 4.20% crude lipid), or 3) H (15.7% crude lipid). Analysis of the litter crude lipid content was conducted before the experiment by using fresh substrate. The L substrate contained 38.0% cornstarch, which was replaced by cellulose (23.0%) and soybean oil (15.0%) in the H food particles. Crude fiber content was 2.56% in the L substrate, 2.95% in the N substrate, and 25.5% in the H substrate. Dry matter was 88.3% in the L substrate, 86.5% in the N substrate, and 90.5% in the H substrate. Metabolizable energy ranged from 10.9 MJ of ME (L), to more than 11.1 MJ of ME in the H substrate, to 11.4 MJ of ME in the N substrate. Crude protein was 16.2% in the N substrate and 16.9% in the L and H substrates. Litter substrates were provided at a height of approximately 15 mm, and they fully covered the floor of each litter tray. Throughout the experiment, the filling level of each tray was controlled on a daily basis, and substrate was added when necessary. Soiled litter substrate was not removed. The proportions of litter particle sizes (approximately 120 g) were examined with the help of a particle sieve at 4 times throughout the trial, and data were averaged for each substrate (Figure 1). Samples were taken immediately before substrates were distributed on the trays (3 samples, wk 20). Throughout the experiment, a sample of used substrate was taken from each tray in wk 26 on the first day of the second video observation (36 samples) and in wk 27 and 29 immediately after video observations had been conducted (111 samples in total). The density of substrates was estimated by weighing 1 L of fresh particles per substrate (L: 792.9 g/L; N: 741.3 g/L; F: 443.6 g/L).

Behavioral Observations

Each compartment was equipped with 2 video cameras installed at a height of approximately 2 m. Hens were marked with a plastic label on the back and could be individually recognized. After a habituation period of 2 wk, in which layers had free access to the 3 different dustbathing trays, video observations were conducted in wk 23, 26, and 29 over 2 d each. Video recordings were analyzed using Observer software (version 5.0, Noldus Information Technology BV, Wageningen, the Netherlands).

The number of DB in the 3 different litter substrates, sham DB on the plastic grid floor (**G**), and the occurrence of foraging behavior (scratching and pecking) were counted at 1-h intervals during the light period by taking an instantaneous scan from each compartment every hour. Dustbath durations (time spent dustbathing) in the L, N, and H treatments were recorded continuously for each individual hen during the light period of each observation day. Duration was not recorded for substrate G. The beginning of a DB was defined when the first vertical wing shake occurred. The preceding bill-raking bouts and ground scratching were not considered. A single DB ended when hens showed a full-body wing shake or interrupted their dustbathing behavior for more than 10 min. Dustbathing behavior that was broken by shorter intervals was considered 1 uninterrupted DB. This definition was adopted from de Jong et al. (2007) and van Liere et al. (1990) and was

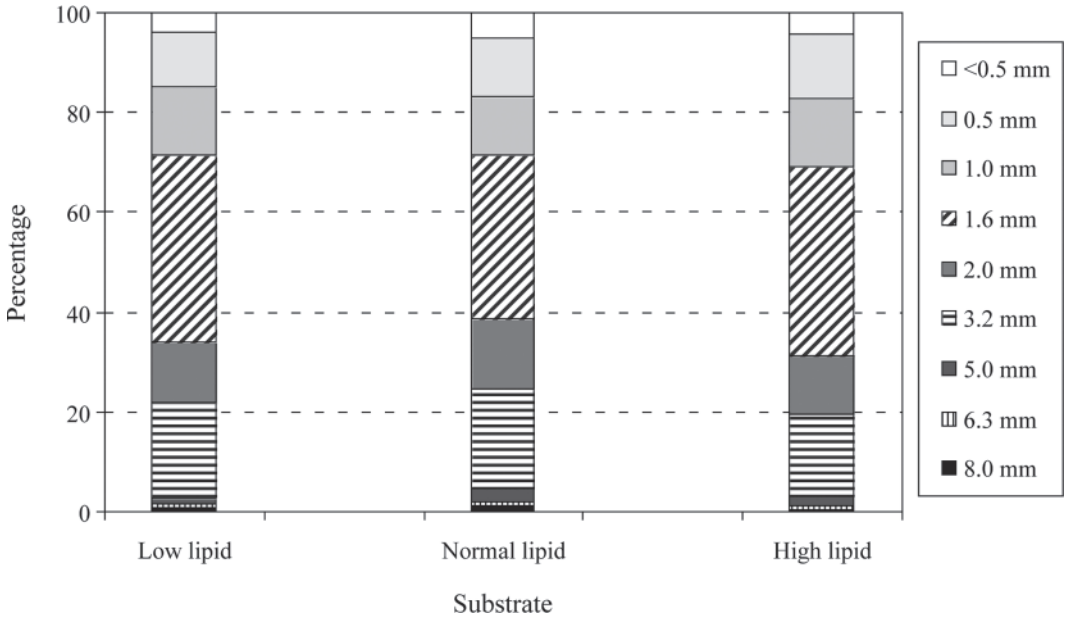

Figure 1. Mean percentage of particle size fractions (mm) of low-lipid (0.82% lipid), normal-lipid (4.2% lipid), and high-lipid (15.7% lipid) food particles.

slightly modified because these authors used an interval of 15 min. From this data set, average durations of DB ($\mathbf{DB_{gross}}$) in the L, N, and H treatments were calculated.

The relative frequencies and durations of the different behavioral patterns within a single DB were analyzed by focal animal sampling, based on a different data set. For each litter tray of the 12 compartments and for each observation day, a 5-h time frame was scheduled, beginning 5 h after the light had been switched on, which resulted in 216 time frames (12 compartments × 3 trays × 6 observation days). During each time frame, the first hen from each compartment entering the observed litter tray and beginning to dustbathe was chosen as the focal bird, provided it had not been chosen as a focal bird at a previous time or in a different litter tray. If a hen had already been observed, the second hen entering the particular tray was chosen, provided it had not served as focal bird before, and so on. Only 1 focal hen per litter tray was observed on each of the 6 observation days. Because the same focal birds were not observed repeatedly, a maximum of 72 focal bird observations (corresponding to the number of hens used in the experiment) were possible. Sixty-five analyses were finally included in the data set because of 7 hens refusing to dustbathe during the 216 time frames set. From this data set, the behavioral patterns "sitting in substrate," "head or side rubbing," and "lying on the side" were recorded as continuous events (state sampling) and the traits "vertical wing shake," "scratching," "bill raking," "pecking in plumage," and "body wing shake" were recorded as events (event sampling). Single behaviors were defined according to the methods of Kruijt (1964) and van Liere (1992a). Relative frequencies and durations of single behaviors within a DB were calculated by dividing the frequency or duration of a single behavior by the overall length of dustbathing. Additionally, the average duration of a single DB in the L, N, and H treatments was calculated by adding the absolute durations of the behaviors recorded by continuous sampling (sitting in substrate, head or side rubbing, lying on the side) and dividing this sum by the number of hens performing the DB in the particular substrate. This method allowed for a precise calculation of average DB durations because only the time periods during which single dustbathing behaviors occurred (continuous states) and during which the plumage of the hens was in direct contact with the particular litter substrate were considered ($\mathbf{DB_{net}}$).

Statistical Analysis

Data on the number of DB, foraging, time spent dustbathing, and average duration of DB_{gross} were analyzed by using a generalized linear mixed model (SAS Institute, 2004; Shabenberger, 2005) and by incorporating adjusted covariance matrices for age and for substrate within pen as repeated measures. The model included the substrate [L, N, H, G (G was recorded only for the variable number of DB)], genotype (LB, LSL), age (23, 26, 29 wk), and the interaction between substrate and genotype (except for the average duration of DB_{gross}) as fixed effects. The gamma distribution with a logarithmic link function was used for the variables number of DB and foraging. The normal distribution with an identity link function was used for the variables time spent dustbathing and average duration of DB_{gross}.

Data on the relative frequency and relative durations of behavioral patterns within a DB, and data on the average duration of DB_{net} were averaged over ages and

otherwise were analyzed as described above. The initial model included substrate (L, N, H) and genotype (LB, LSL) as fixed effects and substrate within pens as repeated measures. Because genotype did not have a significant effect, this effect was removed from the model. The interaction between substrate and genotype was not included because of too few data values. The normal distribution with the identity link function was used for all variables in this data set.

RESULTS

Number of DB

The number of DB was significantly influenced by litter substrate ($F_{3,40} = 45.39$, $P < 0.001$). The highest numbers of DB occurred on the L substrate, and differences in the N, H, and G treatments were significant (all $P < 0.001$; Table 1). The number of DB tended to be higher in the G compared with the H treatment ($P = 0.062$). In addition, the number of DB tended to be higher in the N compared with the H treatment ($P = 0.056$). No effect was found for age ($F_{2,94} = 1.13$, $P = 0.327$), genotype ($F_{1,40} = 2.15$, $P = 0.150$), and the interaction between genotype and substrate ($F_{3,40} = 1.97$, $P = 0.133$).

Time Spent Dustbathing

Time spent dustbathing was affected by substrate ($F_{2,47} = 32.0$, $P < 0.001$), age ($F_{2,47} = 4.92$, $P = 0.012$), and the interaction between substrate and genotype ($F_{2,47} = 3.36$, $P = 0.043$). No effect was found for the single effect of genotype ($F_{1,47} = 0.02$, $P = 0.902$). Analyses within genotype showed that hens from both genotypes spent a longer time dustbathing in the L compared with the N and H substrates ($P < 0.001$; Figure 2). Furthermore, LSL hens spent a longer time dustbathing in the L substrate compared with LB layers ($P = 0.024$), whereas no difference was found between the time the 2 genotypes spent dustbathing in the N ($P = 0.119$) and H ($P = 0.989$) substrates. Time spent dustbathing increased from wk 23 to 26 ($P = 0.004$) and from wk 23 to 29 ($P = 0.045$), whereas no differences occurred between wk 26 and 29 ($P = 0.383$; Table 1).

Average Duration of a Single DB_{gross}

The average duration of single DB_{gross} was not affected by substrate ($F_{2,21} = 0.13$, $P = 0.876$), age ($F_{2,28} = 3.02$, $P = 0.065$), or genotype ($F_{1,21} = 3.31$, $P = 0.083$; Table 1).

Foraging

Foraging was affected by substrate ($F_{3,40} = 71.32$, $P < 0.001$) and the interaction between substrate and genotype ($F_{3,40} = 6.23$, $P = 0.001$), but not by genotype ($F_{1,40} = 3.11$, $P = 0.086$) and age ($F_{2,94} = 0.99$, $P = 0.375$; Table 1). Most foraging behavior was observed in the N treatment, with differences between the L, H, and G treatments being significant (all $P < 0.001$; Table 1). In addition, more foraging incidences occurred in the L treatment in comparison with the G ($P < 0.001$) and H ($P = 0.049$) treatments, and in the H compared with the G ($P < 0.001$) treatment. Despite the significant interaction between substrate and genotype ($F_{3,40} = 6.23$, $P = 0.001$), both genotypes reacted quite similarly in the F, N, and H treatments. However, relative more foraging was performed by LSL hens in the G treatment compared with LB hens ($P < 0.001$; Figure 3).

Behavioral Patterns Within a DB

The single behaviors scratching ($F_{2,18} = 2.39$, $P = 0.120$), bill raking ($F_{2,18} = 1.91$, $P = 0.176$), and pecking in plumage ($F_{2,18} = 0.97$, $P = 0.400$) were not affected by substrate. Substrate affected the number of body wing shakes ($F_{2,18} = 3.56$, $P = 0.050$), with more body wing shakes in the L compared with the N (P

Table 1. Number of dustbaths (DB; least squares means, number of DB/pen), time spent dustbathing (least squares means, min/hen per day), duration of DB including interruptions of up to 10 min (DB_{gross}; least squares means, min/DB), and frequency of hens foraging (least squares means, incidences/pen) as affected by substrate, age, and genotype

	Substrate[1]				Age (wk)			Genotype[2]	
Behavior	Low lipid	Normal lipid	High lipid	Grid floor	23	26	29	LSL	LB
Number of DB (no./pen)	1.19[a]	0.24[b]	0.03[b]	0.24[b]	0.43	0.50	0.35	0.48	0.37
Time spent dustbathing[3] (min)	13.2[a]	3.99[b]	2.79[b]	—	4.27[c]	8.50[ab]	7.18[b]	6.56	6.73
Duration of DB_{gross} (min/DB)	15.7	17.0	15.7	—	19.6[a]	14.9[ab]	13.9[b]	14.2	18.1
Foraging[4] (no./pen)	2.47[b]	3.33[a]	2.16[c]	1.11[d]	2.36	2.27	2.17	2.36	2.17

[a–d]Different letters in a row (category) indicate a significant difference ($P < 0.05$).

[1]Low lipid = feed holding 0.82% lipid (L); normal lipid = feed holding 4.2% lipid (N); high lipid = feed holding 15.7% lipid (H).

[2]LSL = Lohmann Selected Leghorn; LB = Lohmann Brown.

[3]A significant interaction was found between substrate and genotype.

[4]A significant interaction was found between substrate and genotype.

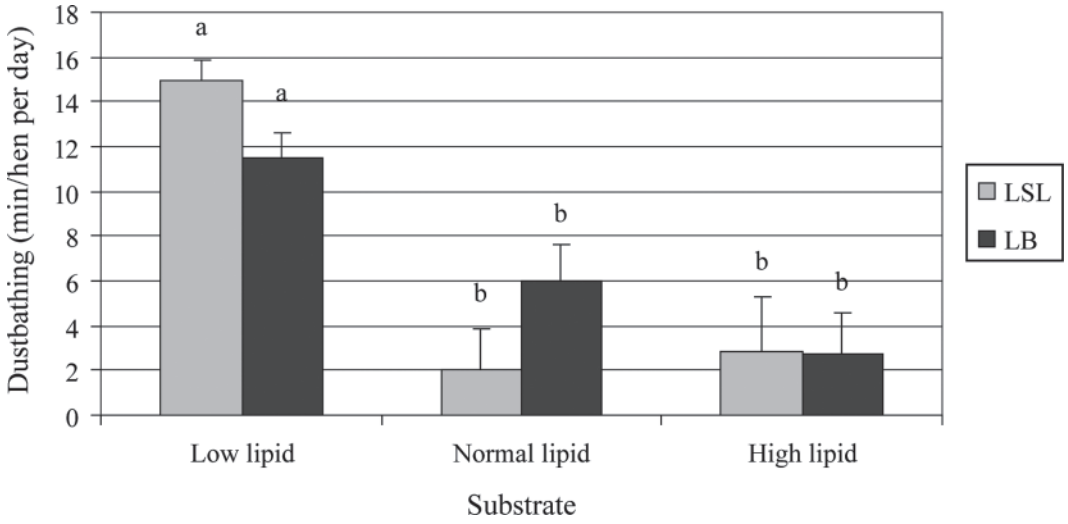

Figure 2. Least squares means ± SE for time spent dustbathing (min/hen per day). Different letters within a genotype (a, b) indicate significant differences ($P < 0.05$) within this genotype. LSL = Lohmann Selected Leghorn; LB = Lohmann Brown; low lipid = feed holding 0.82% lipid; normal lipid = feed holding 4.2% lipid; high lipid = feed holding 15.7% lipid.

= 0.034) treatment. In addition, hens tended to perform more body wing shakes in the L compared with the H treatment ($P = 0.062$). With regard to vertical wing shakes, the overall effect of substrate tended to be significant ($F_{2,18} = 3.10$, $P = 0.070$), and pair-wise comparisons showed more vertical wing shakes in the N compared with the H treatment ($P = 0.024$; Table 2).

Average Duration of a Single DB_{net}

The average duration of a single DB_{net} was affected by substrate ($F_{2,18} = 4.57$, $P = 0.025$), with longer average durations of DB_{net} in the H compared with the N ($P = 0.024$) and L ($P = 0.009$) treatment. No difference in the average duration of DB_{net} was found between the L and N treatments ($P = 0.812$; Table 2).

DISCUSSION

Our results clearly showed that the lipid content of a substrate affected the preference of layers for a particular dustbathing material and altered their dustbathing behavior.

Preferences for DB Substrates

The L substrate was clearly preferred over all other substrates. Furthermore, the preference for substrates with lipid contents conforming to either an N or an H layer diet were very low and were comparable with that of the particle-free G. In a preference test by Scholz et al. (2010), layers were offered wood shavings, a wood-derived material called lignocellulose, an Astroturf mat,

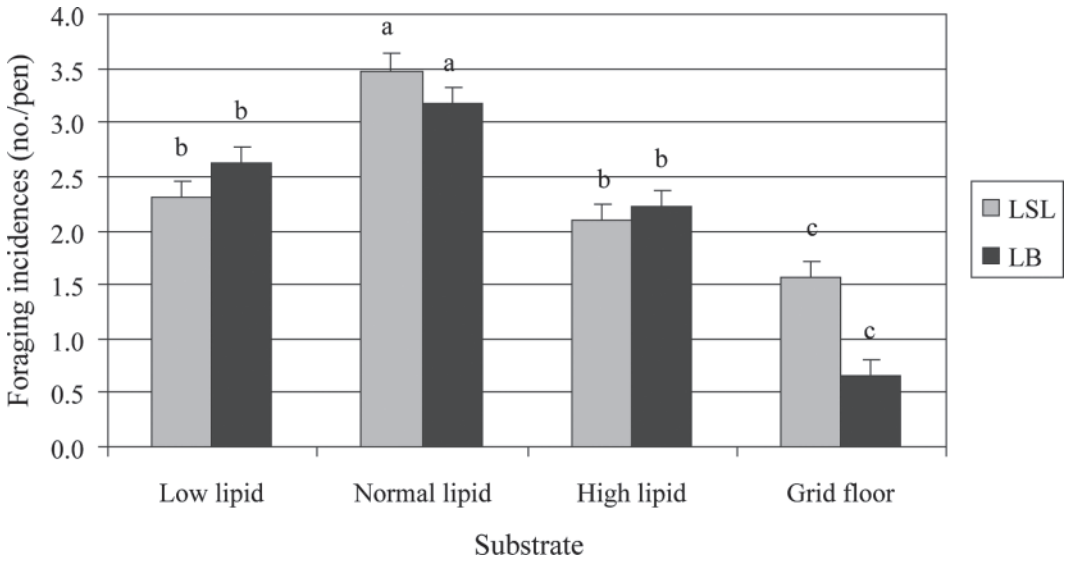

Figure 3. Least squares means ± SE of incidences of foraging per pen. Different letters within a genotype (a–c) indicate significant differences ($P < 0.05$) within this genotype. LSL = Lohmann Selected Leghorn; LB = Lohmann Brown; low lipid = feed holding 0.82% lipid; normal lipid = feed holding 4.2% lipid; high lipid = feed holding 15.7% lipid.

Table 2. Single behaviors within a dustbath (DB)[1]

Variable	Substrate[2]			*P*-value
	Low lipid	Normal lipid	High lipid	
Vertical wing shakes (no.)	16.1[ab]	17.8[a]	14.2[b]	0.070
Scratching (no.)	33.7[a]	32.9[ab]	27.8[b]	0.120
Bill raking (no.)	21.9	24.8	30.1	0.176
Pecking in plumage (no.)	1.74	2.65	1.96	0.340
Body wing shakes (no.)	0.688[a]	0.340[b]	0.399[ab]	0.050
Head or side rubbing (%)	2.57	2.32	2.35	0.234
Lying on the side (%)	52.6	48.8	46.4	0.444
Sitting in substrate (%)	34.31	41.06	43.19	0.164
Average duration of DB_{net} (min/DB)	14.9[b]	15.8[b]	26.4[a]	0.025

[a,b]Different letters indicate a significant difference ($P \leq 0.05$).

[1]Least squares means of relative frequencies (no.) and relative durations (%) of behavioral patterns within a DB and of average duration of DB excluding interruptions of up to 10 min (DB_{net}; min/DB) as affected by substrate.

[2]Low lipid = feed holding 0.82% lipid; normal lipid = feed holding 4.2% lipid; high lipid = feed holding 15.7% lipid.

and food particles (N layer diet) as dustbathing materials. The lignocellulose and wood shavings were preferred over food particles. It was assumed that the lipid content of food (4.20%) may have prevented the hens from using the food particles for dustbathing compared with the almost lipid-free lignocellulose and wood shavings because lipid removal from the plumage of layers may have been inhibited in a substrate having a certain lipid content itself. In the present study, again hens clearly preferred the litter substrate with the lowest lipid content for dustbathing, thus confirming the hypothesis that the lipid content of a substrate plays an important role in the preference of layers for a particular dustbathing material. Hen age did not affect substrate preferences for DB, which suggests that information about lipid content was acquired during the preceding 3 wk of the habituation period. van Liere (1992a) suggested that the pecking experience is important for the selection of a suitable dustbathing substrate. Furthermore, functional experience, such as an adequate reduction of feather lipids in a particular substrate, was suggested as accounting for the development of a particular dustbathing litter preference. Thus, lipid removal in the preceding DB may have influenced substrate preference in the following DB (van Liere, 1992a). Based on our results, a functional experience, gained by successful lipid removal from the plumage during a DB in the almost lipid-free L substrate, seemed to account for the development of a clear preference for this particular substrate. It was surprising that the preference for sham DB on the wire floor was comparable with that of the H substrate, although it contained no friable particles normally needed to trigger dustbathing behavior (Petherick et al., 1995). However, according to a study by Olsson et al. (2002), these findings may possibly be due to an effect of the early rearing experience. Hens in the present experiment were reared without litter substrate until wk 18 of life, and sham dustbathing might have become a habit during this period. In other studies, however, hens clearly preferred friable substrates or Astroturf mats over conventional wire, suggesting that plain wire floorings are not attractive for dustbathing (Merrill et al., 2006; Scholz et al., 2010).

The 3 different substrates were almost identical in color, and the analysis of particle sizes showed only marginal differences in particle size fractions among the L, N, and H treatments. However, the L treatment was more adherent than the N and H treatments. Because of these minor differences in consistency, the influence of substrate texture or appearance was considered to be small but cannot be entirely excluded.

Time Spent Dustbathing and Average Duration of DB

The high number of DB in the L compared with the N and H treatments corresponded to the longest time spent dustbathing observed in this substrate. When only dustbathing sequences in which the plumage of the hens was in direct contact with the substrate were considered to calculate the average DB_{net} (sitting in substrate, head or side rubbing, lying on the side), the average durations of DB were longer in the H compared with the L and N treatments. Following our assumption that a high lipid substrate has a limited effect on lipid removal and may even contribute to further accumulation of feather lipids, the longer average duration of DB_{net} in the H treatment (15.7% crude lipid) could have been due to unsuccessful attempts of layers trying to remove the feather lipids. However, differences in lipid content between the L and N treatments (0.82 vs. 4.20% crude lipid) did not lead to differences in the average duration of DB_{net}. The difference in lipid contents between the L and N treatments might have been too small for the birds to realize it. In a prior experiment, DB in food particles were longer compared with those in almost lipid-free lignocellulose and wood shavings substrates, supporting the idea that the lipid content of a substrate may extend the average duration of DB because of its possible inadequate function of stale lipid removal from the plumage of hens (Scholz et al., 2010). Hens may simply need more time to get rid

of excess lipids, and in substrates with lipid contents over a certain threshold, this might not be possible at all. In accordance with our findings, van Liere et al. (1990) found longer DB in wood shavings compared with sand, and they suggested that wood shavings might have been less effective in removing feather lipids and therefore DB might have lasted longer.

Behavioral Patterns Within a DB

With regard to single behaviors within a DB, hens showed more body wing shakes in the L compared with the N and H treatments. Body wing shakes serve to remove substrate from the plumage and therefore occur mostly at the end of a single DB (van Liere, 1992a). A higher number of body wing shakes in the more adherent L substrate might have been necessary to remove the L substrate from the feathers of layers. Because the N and H substrates may have inhibited the removal of stale lipids or may even have led to further lipid accumulation, DB may not have been sensed as complete, which would lead to fewer final body wing shakes. Rubbing behavior intensifies the contact between the litter and proximal integument. It consists of lying on the side and head or side rubbing (van Liere, 1992b) and did not differ among the L, N, and H treatments. Because of their similar particle size fractions, it can be assumed that the different substrates may have similarly penetrated the plumage up to the skin and possibly either spread (N, H) or removed (L) lipids there.

Foraging

Although the N substrate was freely available at the feed troughs, in the litter trays it was highly preferred for foraging, followed by the L, H, and G substrates, with the latter being the least attractive. Weeks and Nicol (2006) described foraging as being unaffected by the provision of food, thus suggesting a different motivation for this behavioral trait compared with food consumption.

Conclusions

Our results show that preference for a dustbathing substrate increases with decreasing lipid content. In the present study, there seemed to be a threshold around 4% lipid at which the preference for that level and above was dramatically reduced. In addition, feed conforming to a normal layer diet with 4.20% lipid (N), which is commonly provided as the litter substrate in furnished caged housing systems, was found to be less attractive for dustbathing, equal to the preference for G. Furthermore, the average duration of DB increased in substrates containing a higher lipid content, possibly because of the unsuccessful attempts of hens to remove stale lipids from their plumage. Thus, our results suggest that food particles may not be a suitable dustbathing substrate compared with friable L substrates and therefore do not seem to fulfill the requirements of the European Union regulation with respect to satisfying the ethological needs of hens.

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REFERENCES

de Jong, I. C., M. Wolthuis-Fillerup, and C. G. van Reenen. 2007. Strength of preference for dustbathing and foraging substrates in laying hens. Appl. Anim. Behav. Sci. 104:24–36.

European Union. 1999. Council Directive 1999/74/EC. Laying down minimum standards for the protection of laying hens. Off. J. L 203:54–55.

Kruijt, J. P. 1964. Ontogeny of social behaviour in Burmese red junglefowl (*Gallus gallus spadiceus*) Bonnaterre. Behavior 12(Suppl.):1–201.

Merrill, R. J. N., J. J. Cooper, M. J. Albentosa, and C. J. Nicol. 2006. The preference of laying hens for perforated Astroturf over conventional wire as a dustbathing substrate in furnished cages. Anim. Welf. 15:173–178.

Olsson, I. A. S., L. J. Keeling, and I. J. H. Duncan. 2002. Why do hens sham dustbathe when they have litter? Appl. Anim. Behav. Sci. 76:53–65.

Petherick, J. C., E. Seawright, D. Waddington, I. J. H. Duncan, and L. B. Murphy. 1995. The role of perception in the causation of dustbathing behavior in domestic fowl. Anim. Behav. 49:1521–1530.

SAS Institute. 2004. SAS OnlineDoc 9.1.3. SAS Inst. Inc., Cary, NC.

Scholz, B., S. Urselmans, J. B. Kjaer, and L. Schrader. 2010. Food, wood or plastic as substrates for dustbathing and foraging in laying hens: A preference test. Poult. Sci. 89:1584–1589.

Shabenberger, O. 2005. Introducing the GLIMMIX procedure for generalized linear mixed models. Paper 196–30 in Proc. 30th SAS Users Group Int. Conf., Philadelphia, PA. SAS Inst. Inc., Cary, NC.

van Liere, D. W. 1992a. The significance of fowls' bathing in dust. Anim. Welf. 1:187–202.

van Liere, D. W. 1992b. Dustbathing as related to proximal and distal feather lipids in laying hens. Behav. Processes 26:177–188.

van Liere, D. W., S. E. Aggrey, F. M. R. Brouns, and P. R. Wiepkema. 1991. Oiling behaviour and the effect of lipids on dustbathing behaviour in laying hens *Gallus gallus domesticus*. Behav. Processes 24:71–81.

van Liere, D. W., and S. Bokma. 1987. Short-term feather maintenance as a function of dust-bathing in laying hens. Appl. Anim. Behav. Sci. 18:197–204.

van Liere, D. W., J. Kooijman, and P. R. Wiepkema. 1990. Dustbathing behaviour of laying hens as related to quality of dustbathing material. Appl. Anim. Behav. Sci. 26:127–141.

Weeks, C. A., and C. J. Nicol. 2006. Behavioural needs, priorities and preferences of laying hens. World's Poult. Sci. J. 62:296–307.