Project no. SSPE-CT-2004-502315

Project acronym: LAYWEL

Project title
Welfare implications of changes in production systems for laying hens

Specific Targeted Research Project

Thematic Priority: Integrating and strengthening the ERA, Area 8.1.B.1.4, task 7

Deliverable 4.4
Definition of behavioural indicators to evaluate substrate quality

Due date of deliverable: 7
Actual submission date: 10

Start date of project: 01/01/2004 Duration: 24 month

Organisation name of lead contractor for this deliverable: ID-Lelystad

Revision [final]

Project co-funded by the European Commission within the Sixth Framework Programme (2002-2006)		
Dissemination Level		
PU	Public	
PP	Restricted to other programme participants (including the Commission Services)	x
RE	Restricted to a group specified by the consortium (including the Commission Services)	
CO	Confidential, only for members of the consortium (including the Commission Services)	

Definition of behavioural indicators to evaluate substrate quality in different housing systems for laying hens

Ingrid de Jong and Maaike Fillerup

Animal Sciences Group of Wageningen UR, Division Animal Resources Development, Animal Welfare Group, PO Box 65, 8200 AB Lelystad, The Netherlands

1. Introduction and aim

In task 4.5 substrate quality will be evaluated in different housing systems for laying hens. The objective of task 4.4 is to develop behavioural criteria to evaluate the substrate quality in different housing systems for laying hens. Criteria will be developed using two approaches:

- A short literature overview. Literature on problems associated with quality or availability of substrate will be reviewed (2.).
- An experimental approach (3.). Hens will be housed on different types of substrate of which we assume that are preferred or not preferred to perform dustbathing or foraging behaviour. Behavioural observations of the hens on different substrate types will be used to define criteria to assess substrate quality.

A report of the results of above mentioned approaches will be followed by a list of parameters that will be used in task 4.5 (4.).

2. Short literature overview

2.1. Substrate in laying hen housing systems

A number of studies have investigated substrate-directed behaviour in laying hens, in particular dustbathing (e.g., Vestergaard, 1982; Vestergaard et al., 1990; Van Liere, 1991; Vestergaard and Hogan, 1992; Vestergaard et al., 1997; Widowski and Duncan, 2000; Olsson et al., 2002a; Olsson and Keeling, 2002; Olsson et al., 2002b). It has been shown that domestic hens are attracted to substrate and show a preference for substrate as a floor type (Dawkins, 1981; Dawkins, 1983; Matthews et al., 1995). Hens housed in battery cages are deprived of substrate and it has been shown that this increases the risk of outbreaks of feather pecking and cannibalism (e.g., Huber-Eicher and Wechsler, 1997; 1998; Wechsler and Huber-Eicher, 1998; Aerni et al., 2000; El-Lethey et al., 2000; Klein et al., 2000; El-lethey et al., 2001; Nicol et al., 2001; Olsson and Keeling, 2002) and sham dustbathing may be observed (Van Liere and Wiepkema, 1992; Larsen et al., 2000; Widowski and Duncan, 2000).

The Council Directive 1999/74/EC laying down minimum standards for the protection of laying hens states that hens should be housed in enriched cages or alternative systems from 2012 onwards. Alternative systems should have at least 250 cm^2 area with substrate per hen and in enriched cages hens should be provided with substrate such that pecking and scratching are possible (CEC, 1999). However, especially in enriched cages the substrate area is a point of discussion as it is often a small area and only small amounts of substrate are provided (Appleby et al., 2002). It is seriously questioned if this substrate area fulfils the need of the hens with respect to substrate related behaviours like foraging and dustbathing. Sham dustbathing is often seen and it does not reduce the motivation to dustbathe in litter (Olsson et al., 2002b) which may suggest that the litter as provided in enriched cages does not fulfil the needs of the hens. In addition it has been observed that hens in enriched cages have a low

motivation for using the litter areas to dustbath (Olsson and Keeling, 2002). In a large study on different types of enriched cages it was observed that the substrate areas were frequently used but not as much as in alternative housing systems (Appleby et al., 2002).

Although the substrate area per bird in alternative systems (aviaries, free range systems, organic systems) is much larger as compared to enriched cage systems it has been questioned if these litter areas do fulfil the needs of the hens. Abnormal behaviour related to the availability or quality of substrate has also been observed in these systems, like feather pecking and sham dustbathing (Fiks, personal communication; Oden et al., 2002). Oden et al. (2002) concluded that the substrate area was insufficient in two types of aviary systems. They observed a high frequency of aggressive pecks in substrate areas, that might be related to the preference of birds to space out when foraging which was not possible due to the limited substrate space per bird (Keeling and Duncan, 1991, in Oden et al., 2002). Litter quality was poor in these aviary systems, especially at the end of the laying period, and fewer birds used the litter for dustbathing when it became less friable (Oden et al., 2002).

2.2. Behaviours associated with availability and quality of substrate

From the huge amount of literature on the causation of feather pecking in domestic fowl it becomes clear that the provision of good foraging material may reduce the prevalence of feather pecking (e.g., Huber-Eicher and Wechsler, 1997; 1998; Wechsler and Huber-Eicher, 1998; Aerni et al., 2000; El-Lethey et al., 2000; El-lethey et al., 2001; Nicol et al., 2001). However, feather pecking may be caused by multiple environmental factors like light intensity, group size and stocking density, food form and rearing conditions (see e.g. overview in Blokhuis and Wiepkema, 1989). The prevalence of feather pecking may be used as indicator of substrate quality but should therefore be combined with other behavioural measures. In addition to feather pecking the frequency of aggressive pecks in the substrate

area may be related to overcrowding in the litter area (Oden et al., 2002) and may thus be used as criterion to assess substrate quality.

In the absence of suitable substrate for dustbathing sham dustbathing may be observed (Lindberg and Nicol, 1997; Olsson et al., 2002b) which thus may be used as a criterion to assess substrate quality. In addition, as also described in 3.1., in a substrate preferred for dustbathing the duration of a dustbath is longer as compared to substrate less preferred for dustbathing (Van Liere et al., 1990), and there is a greater probability of complete dustbaths (Van Liere et al., 1990; Vestergaard et al., 1990; Van Liere and Siard, 1991).

Laying hens have an innate behavioural rhythm for certain behaviours (like feeding, dustbathing, foraging or perching) (e.g., Oden et al., 2002) and therefore the opportunity to perform these behaviours at the right time may be an important criterion to assess the quality of the substrate area. Under natural conditions laying hens synchronise these behaviours (Mench and Keeling, 2001). Thus, the number of hens performing foraging and dustbathing on the litter area in the preferred periods (middle of the light period for dustbathing and before lights off for foraging, see 3.3) may reflect the accessability of the substrate area in housing systems (Oden et al., 2002).

3. Experimental approach

3.1. Introduction

Numerous studies have focussed on (the development of) dustbathing in domestic fowl. Substrate preferences for dustbathing seem to vary between individuals (Van Liere and Siard, 1991) and may be dependent on previous experience with a particular substrate (Van Liere et al., 1990; Van Liere and Siard, 1991; Vestergaard and Hogan, 1992; Sanotra et al., 1995). However, from the different studies it has become clear that peat (Petherick and Duncan, 1989; Van Liere and Siard, 1991; Matthews et al., 1995) and sand (Van Liere et al., 1990; Vestergaard and Hogan, 1992) are preferred for dustbathing whereas sawdust, woodshavings and straw are not (Petherick and Duncan, 1989; Van Liere et al., 1990; Van Liere and Siard, 1991; Vestergaard and Hogan, 1992; Matthews et al., 1995; Sanotra et al., 1995). It has been shown that dustbaths that are performed on a less preferred substrate like woodshavings are shorter as compared to dustbaths performed on preferred substrates (Van Liere et al., 1990). Moreover, on sand there was a greater likelihood of dustbaths ending with a body shake (Vestergaard et al., 1990), thus, containing all elements of a dustbath (Van Liere, 1991), as compared to dustbaths on less preferred substrates. These data suggest that the quality of a substrate in relation to dustbathing behaviour can be judged by measuring the length of dustbaths and scoring if dustbaths contain all behavioural elements (complete dustbaths) (Van Liere, 1991).

Only few studies have focussed on litter preferences in relation to foraging behaviour. Whereas Matthews et al. (1995) suggest that peat, sand and woodshavings are equally valued for foraging, it has also been suggested that peat (Petherick and Duncan, 1989), woodshavings (Vestergaard and Hogan, 1992) and straw (Sanotra et al., 1995) were preferred for foraging behaviour. Substrate preferences were determined by observing the frequency of pecking and

scratching in the particular substrates, hypothesizing that these behaviours occur more frequently in preferred substrate types.

In the present experiment we housed laying hens on three different types of substrates of which we expect that they are preferred or not preferred for dustbathing, i.e. peat, sand and woodshavings. Although it is more difficult to state that any particular substrate is preferred for foraging, until now it has not been suggested that sand is a preferred substrate for foraging whereas it has been suggested that peat and woodshavings are preferred for foraging. Frequency of foraging as well as frequency, duration and the quality of dustbathing behaviour (does it contain all elements or not) are observed on two subsequent days to determine if these measurements can be used to assess substrate quality in commercial laying hen housing systems.

3.2. Materials and methods

3.2.1. Animals and housing

A total of 10 laying hens (ISA Brown, Verbeek, Putten, The Netherlands) were used. Hens were obtained from a commercial farm and reared on battery cages. Hens were 16 weeks of age upon arrival at the experimental farm. Upon arrival, hens were weighed, wingtagged and housed in two groups of four birds in floor pens (1.5 x 1.0 m) with wire floor and four laying nests in one climate controlled room. At 18 weeks of age, hens of one group were individually housed in a floor pen with either peat moss, wood shavings or sand (0.75 x 1.0 m) and a laying nest. The substrate layer was about 5 cm deep. Pens were separated with a wire mesh and were in the same room, thus hens could have visual and auditory contact with other hens. After one week the hens were rehoused in floor pens with another type of substrate. After three weeks, hens had been housed on all three different types of substrate.

Sequence of substrates was randomised per hen. Food and water were always available *ad libitum*. Lights were on from 02.00 h – 18.00 h. After the test period, hens were relocated for use in another experiment and a new group of four hens (at this time 22 weeks of age) was housed on the different substrate types. Pens were cleaned and new substrate was provided when a new group of hens was introduced in the substrate pens. The same procedure was followed for one more batch of two hens (aged 26 weeks at the start of the experiment).

3.2.2. Behavioural observations

Behaviour of the hens was recorded on videotape during the light period on the 6th and 7th day that hens were housed on the particular substrates. The frequency and duration of dustbathing behaviour on these days was recorded using the Observer software (version 4.1, Noldus, Wageningen, The Netherlands). 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). Dustbathing behaviour was scored as either complete dustbathing behaviour or incomplete dustbathing behaviour as defined earlier (Vestergaard et al., 1990; Van Liere, 1991). A complete bout of dustbathing contained all elements of a typical bout as described earlier, that is vertical wingshaking, scratching, bill raking, head rubbing and side rubbing whereas incomplete bouts lacked one or more elements (Vestergaard et al., 1990; Van Liere, 1991).

Scans of the tapes showed that foraging behaviour was most frequently observed just after lights on, in the middle of the light period and just before lights of. Therefore, foraging behaviour was scored during the following periods: 02.00 – 04.00 h, 09.00 – 13.00 h, 16.00 – 18.00 h on both days by 0/1 sampling every 5 min using the Observer software. This means

that it is scored if foraging was observed (1) or not (0) in each 5-min period. Foraging behaviour includes pecking and scratching at potential food sources with accompanying locomotor activity (Cooper and Albentosa, 2003).

3.2.3. Statistical analysis

Both non-parametric methods (based on rank numbers) and parametric methods have been used. The methods based on rank numbers were more robust (but less efficient and more limited in scope than the parametric methods). The methods based on rank numbers added support to the results from the parametric methods for response variables that were irregular in some aspects. Therefore we only present here the results of the non-parametric analysis.

In the non-parametric analyses, any effects over time, as represented by changes from week to week, were assumed to be negligible. Batch effects were assumed to be negligible as well. A test on day effects (i.e. a difference between the last two days of an experimental week) was performed. Per animal the mean for each day (over the treatments in the 3 weeks) was calculated. Differences between the means per animal were calculated and analysed with Wilcoxon's signed rank test. Hereafter, data are averaged per animal over the two days. Main effects for substrates were studied pairwise. Per animal, the difference in response (expressed over the 3 periods within a day) between two substrates was calculated. These differences were analysed with Wilcoxon's signed rank test. Interaction between substrates and periods (when appropriate) was handled in a similar fashion. Differences per animal between substrates were calculated within periods, say d1 and d2 for periods numbered 1 and 2. For any two periods, say periods numbered 1 and 2, the differences d1- d2 were calculated and analysed with Wilcoxon's signed rank test. Main effects for periods (when appropriate) were studied for pairs of periods by analysing differences within animals averaged over substrates. For those animals where both complete and incomplete bathing occurred, the length of

bathing and the number of times dust bathing occurred were compared by Wilcoxon's signed rank test applied to differences between averages calculated within animals. All analyses were performed with the statistical programming language Genstat (Committee, 2000).

3.3. Results

3.3.1. Dustbathing

Figure 1 shows the total duration of dustbathing per substrate and the mean duration per dustbath per substrate type. The total time spent on dustbathing is significantly higher on woodshavings as compared to sand ($P<0.05$) whereas no differences were found between woodshavings and peat moss and sand and peat moss. However, the mean duration per dustbath did not differ significantly between the different substrates (Figure 1). This can be explained by the fact that the dustbathing frequency is significantly higher in woodshavings as compared to sand ($P<0.01$) (Figure 2). When dustbaths were classified as complete or incomplete, it turned out that there were more incomplete dustbaths in woodshavings as compared to sand ($p<0.01$) (Figure 2). In annex 1 the frequency of dustbathing per duration class and the distribution of dustbathing over the light period is shown. Dustbathing was predominantly observed in the middle of the light period.

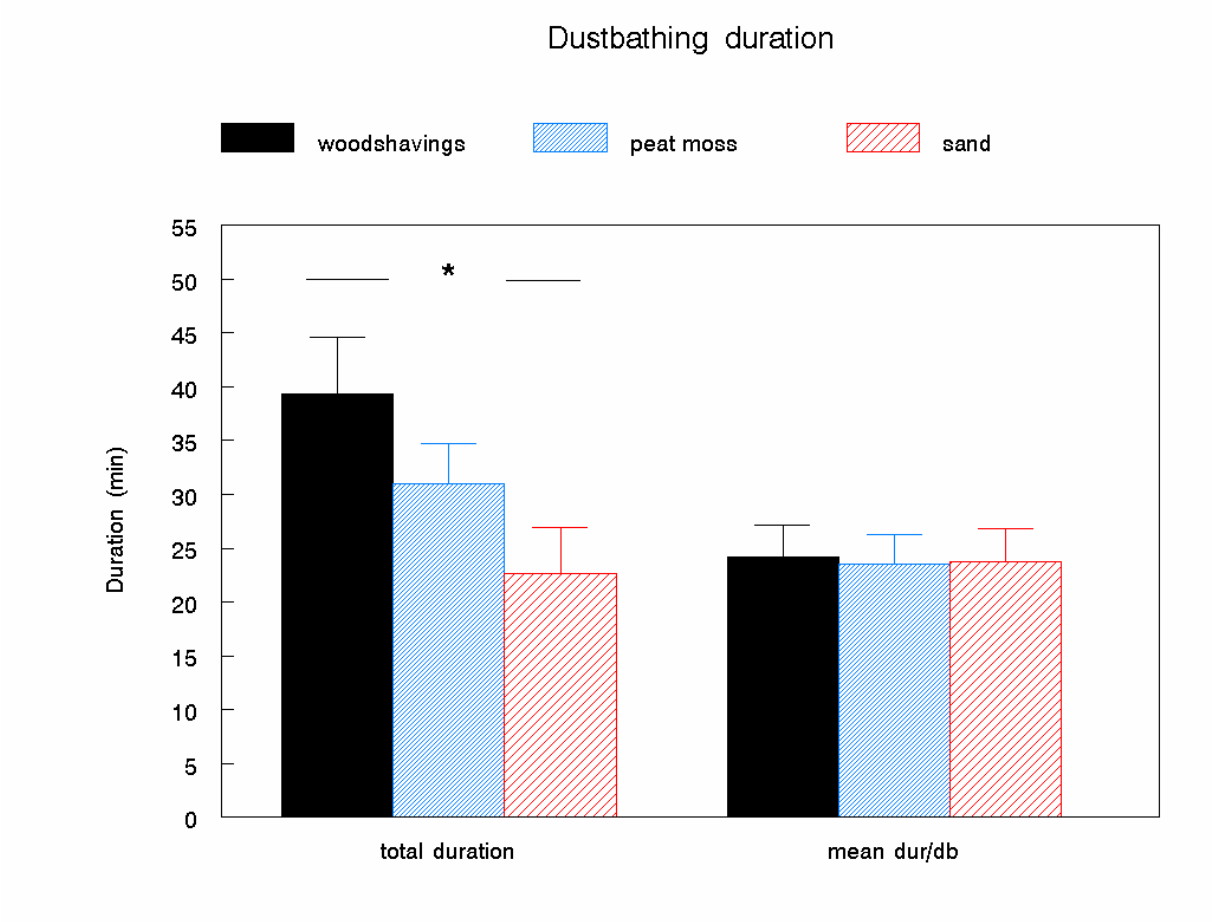

Figure 1. Total duration of dustbathing in the different substrate types, and mean duration per dustbath per substrate type. * $P<0.05$.

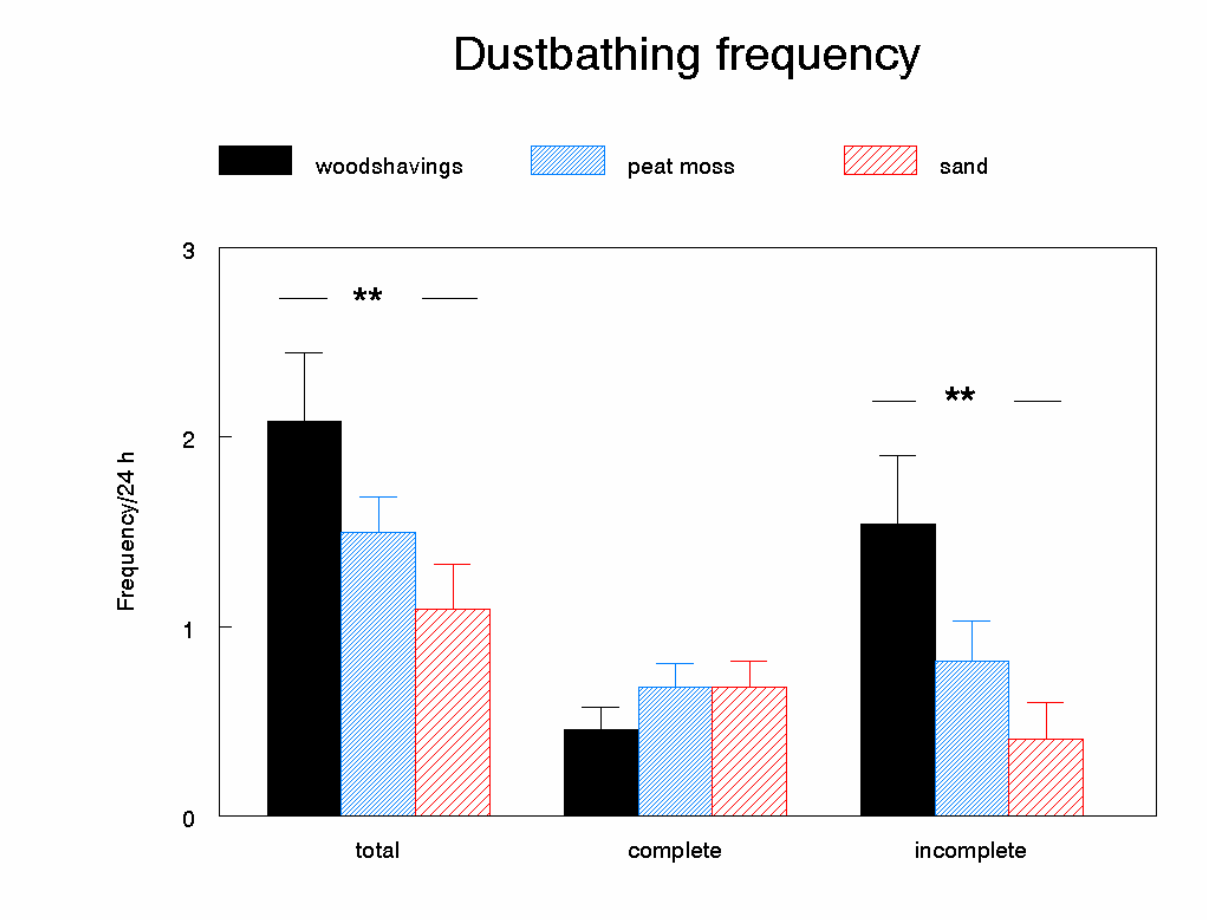

Figure 2. Total frequency of dustbathing per 24 h per substrate type, and frequency per 24 h of complete en incomplete dustbaths per substrate type. ** $P<0.01$.

3.3.2. Foraging

The time spent foraging per observation period are shown in figure 3. A significant effect of the observation period for the time spent on foraging behaviour was found ($p<0.05$). The time spent foraging was highest in the middle of the light period. In addition, an interaction was found between substrate type and observation period ($P<0.05$). In period 1, time spent foraging was higher in peat as compared to woodshavings, whereas in period 2 and 3 the time spent foraging was higher in sand as compared to peat. No significant differences were found in the time spent foraging between substrates within one observation period. Total time spent foraging did not differ between substrates (data not shown).

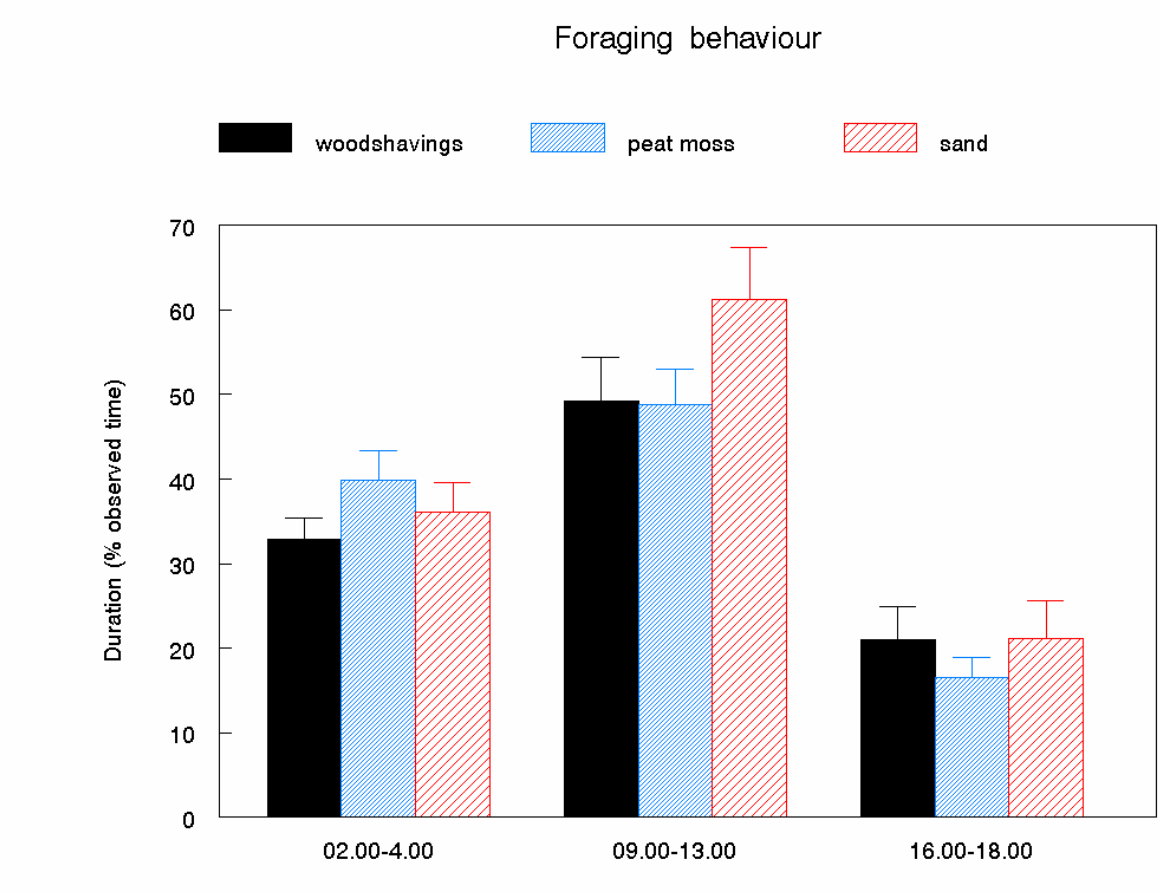

Figure 3. Duration of foraging behaviour expressed as proportion of observed frequency for each observation period.

3.4. Discussion and conclusions

From previous research it was expected that in a substrate that is supposed to be least preferred for dustbathing, i.e. woodshavings (Petherick and Duncan, 1989; Van Liere et al., 1990; Van Liere and Siard, 1991; Vestergaard and Hogan, 1992; Matthews et al., 1995), dustbaths should be shorter as compared to substrates that are supposed to be preferred for dustbathing, i.e. sand or peat (Petherick and Duncan, 1989; Van Liere et al., 1990; Van Liere and Siard, 1991; Vestergaard and Hogan, 1992; Matthews et al., 1995). In addition, it could be expected that complete dustbaths were more frequently observed on substrates that are supposed to be preferred for dustbathing (Van Liere et al., 1990; Vestergaard et al., 1990; Van Liere and Siard, 1991). In this experiment we confirmed these data, as we found that on woodshavings more incomplete dustbaths were performed as compared to sand. The total duration of dustbathing however was higher on woodshavings as compared to sand, which was a result of the fact that dustbathing more frequently occurred on woodshavings but most of these dustbaths were incomplete. It has been suggested from literature that peat and sand are both preferred for dustbathing (Petherick and Duncan, 1989; Van Liere and Siard, 1991; Van Liere and Wiepkema, 1992; Vestergaard and Hogan, 1992; Matthews et al., 1995), and also here we did not observe a difference in time or frequency of dustbathing between these substrates.

With respect to the definition of behavioural criteria for the assessment of substrate quality in relation to dustbathing we suggest from this experiment that dustbathing frequency in combination with the classification as complete or incomplete should give information about substrate quality in laying hen housing systems. From figure B as shown in Annex 1 it can be concluded that dustbathing most frequently occurred in the middle of the light period, which confirms previous suggestions from literature (Vestergaard, 1982). It therefore seems

reasonable to focus on the middle of the light period to study substrate quality with respect to dustbathing.

From literature it could not be concluded that there are clear substrate preferences to perform foraging behaviour although it might be suggested that peat and woodshavings are preferred over sand (Vestergaard and Hogan, 1992; Matthews et al., 1995; Petherick et al., 1995; Sanotra et al., 1995). Here we did not find differences in time spent foraging on the different substrate types, which confirms the earlier suggestion. Surprisingly, more foraging behaviour occurred during the middle of the light period, whereas from literature it has been suggested that foraging frequency is highest just before the dark period (Savory et al., 1978; De Jong et al., submitted). This could be explained by the fact that scratching may be related to the performance of dustbathing (Van Liere, 1991; Vestergaard and Hogan, 1992) and here we could not clearly distinguish if foraging behaviour belonged to the initiation phase of dustbathing. With respect to the development of behavioural criteria to assess substrate quality in relation to foraging behaviour we suggest to observe the frequency of foraging behaviour, as in literature it has been suggested that this is related to substrate preference to perform this behaviour (Petherick and Duncan, 1989; Petherick et al., 1990; Matthews et al., 1995). In addition we suggest to observe foraging behaviour when it will most frequently occur and might not be associated with dustbathing, i.e. at the end of the light period.

4. Behavioural criteria to assess substrate quality in different laying hen housing systems

Combining the results of the literature overview and the experimental approach we suggest the following criteria to assess substrate quality in different laying hen housing systems:

1. **Dustbathing:**

- number of hens dustbathing;
- whether the dustbaths are complete or incomplete, using the definitions as decribed in 3.2.2. (using focal animal sampling);
- duration of dustbathing (using focal animal sampling);
- number of hens sham dustbathing;
- number of dustbathes that are disturbed (e.g, by other hens or feeders that run).

Observation period: middle 4 h of the light period.

2. **Foraging and pecking behaviour:**

- number of hens showing foraging behaviour in the substrate area;
- frequency of aggressive pecking in the substrate area;
- frequency of feather pecking.

Observation period: 2 h before lights off.

3. **Other parameters**

- substrate type;
- substrate quality (i.e., particle size, %dry matter, loose structure or compacted, litter thickness in cm);

- system characteristics: type, manufacturer, housing density, substrate area, size and shape of entrance to litter area, accessibility of litterbox.

Acknowledgements

This work was financially supported by the European Commission, within the 6th Framework Programme, contract No. SSPE-CT-2004-502315 and the Dutch Ministry of Agriculture, Nature and Food Quality. The text represents the authors' views and does not necessarily represent a position of the Commission who will not be liable for the use made of such information. Thea Fiks is acknowledged for her comments on the draft report.

References

Aerni, V., H. El-Lethey, and B. Wechsler. 2000. Effect of foraging material and food form on feather pecking in laying hens. Br. Poultry Sci. 41: 16-21.

Appleby, M. C., A. W. Walker, C. J. Nicol, A. C. Lindberg, R. Freire, B. O. Hughes, and H. A. Elson. 2002. Development of furnished cages for laying hens. British Poultry Science 43(4): 489-500.

Blokhuis, H. J., and P. R. Wiepkema. 1989. Studies of feather pecking in poultry. Veterinary Quarterly 20: 6-9.

Committee, Genstat (2000). The guide to GenStat. *In* R. W. Payne (Ed.). VSN International, Oxford.

CEC (Commission of the European Communities). 1999. Council directive 1999/74/EC of 19 july 1999 laying down minimum standards for the protection of laying hens. Official journal of the European Communities L203: 53-57.

Cooper, J. J., and M. J. Albentosa. 2003. Behavioural priorities of laying hens. Avian and Poultry Biology Reviews 14(3): 127-149.

Dawkins, M. 1981. Priorities in the Cage Size and Flooring Preferences of Domestic Hens. British Poultry Science 22(3): 255-263.

Dawkins, M. S. 1983. Cage Size and Flooring Preferences in Litter-Reared and Cage-Reared Hens. British Poultry Science 24(2): 177-182.

De Jong, I. C., M. Fillerup, and H. J. Blokhuis. submitted. Effect of scattered feeding and feeding twice a day during rearing on parameters of hunger and frustration in broiler breeders. Appl. Anim. Behav. Sci.

El-Lethey, H., V. Aerni, T. W. Jungi, and B. Wechsler. 2000. Stress and feather pecking in laying hens in relation to housing conditions. Br. Poultry Sci. 41: 22-28.

El-lethey, H., T. W. Jungi, and B. Huber-Eicher. 2001. Effects of feeding corticosterone and housing conditions on feather pecking in laying hens. Physiol. Behav. 73: 243-251.

Huber-Eicher, B., and B. Wechsler. 1997. Feather pecking in domestic chicks: its relation to dustbathing and foraging. Anim. Behav. 54: 757-768.

Huber-Eicher, B., and B. Wechsler. 1998. The effect of quality and availability of foraging materials on feather pecking in laying hens. Anim. Behav. 55: 861-873.

Klein, T., E. Zeltner, and B. Huber-Eicher. 2000. Are genetic differences in foraging behaviour of laying hen chicks paralleled by hybrid-specific differences in feather pecking? Appl. Anim. Behav. Sci. 70: 143-155.

Larsen, B. H., K. S. Vestergaard, and J. A. Hogan. 2000. Development of dustbathing behavior sequences in the domestic fowl: the significance of functional experience. Dev. Psychobiology 37: 5-12.

Lindberg, A. C., and C. J. Nicol. 1997. Dustbathing in modified battery cages: is sham dustbathing an adequate substrate? Appl. Anim. Behav. Sci. 55: 113-128.

Matthews, L. R., W. Temple, T. M. Foster, A. W. Walker, and T. M. McAdie (1995). Comparison of the demand for dustbathing substrates by layer hens, *29th International Congress of the ISAE*, pp. 11-12.

Mench, J., and L. J. Keeling (2001). The social behaviour of domestic birds. *In* L. J. Keeling and H. W. Gonyou (Eds.), *Social behaviour in farm animals*, pp. 177-209. CABI, Wallingford.

Nicol, C. J., A. C. Lindberg, A. J. Phillips, S. J. Pope, L. J. Wilkins, and L. E. Green. 2001. Influence or prior exposure to wood shavings on feather pecking, dustbathing and foraging in adult laying hens. Appl. Anim. Behav. Sci. 73: 141-155.

Oden, K., L. J. Keeling, and B. Algers. 2002. Behaviour of laying hens in two types of aviary systems on 25 commercial farms in sweden. Br. Poultry Sci. 43: 169-181.

Olsson, I. A. S., I. J. H. Duncan, L. J. Keeling, and T. M. Widowski. 2002a. How important is social facilitation for dustbathing in laying hens? Applied Animal Behaviour Science 79(4): 285-297.

Olsson, I. A. S., and L. J. Keeling. 2002. No effect of social competition on sham dustbathing in furnished cages for laying hens. Acta Agriculturae Scandinavica Section a-Animal Science 52: 253-256.

Olsson, I. A. S., L. J. Keeling, and I. J. H. Duncan. 2002b. Why do hens sham dustbathe when they have litter? Appl. Anim. Behav. Sci. 76: 53-64.

Petherick, J. C., and I. J. H. Duncan. 1989. Behaviour of young domestic fowl directed towards different substrates. Br. Poultry Sci. 30: 229-238.

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. Animal Behaviour 49(6): 1521-1530.

Petherick, J. C., D. Waddington, and I. J. H. Duncan. 1990. Learning to gain access to a foraging and dustbathing substrate by domestic fowl: is out of sight out of mind? Behav. Proc. 22: 213-226.

Sanotra, G. S., K. S. Vestergaard, J. F. Agger, and L. G. Lawson. 1995. The Relative Preferences for Feathers, Straw, Wood-Shavings and Sand for Dustbathing, Pecking and Scratching in Domestic Chicks. Applied Animal Behaviour Science 43(4): 263-277.

Savory, C. J., D. G. M. Wood-Gush, and I. J. H. Duncan. 1978. Feeding behaviour in a population of domestic fowls in the wild. Appl. Anim. Ethology 4: 13-27.

Van Liere, D. W. (1991). Function and organization of dustbathing in laying hens, Landbouwuniversiteit Wageningen (Thesis).

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.

Van Liere, D. W., and N. Siard (1991). The experience with litter and subsequent selection of bathing substrates in laying hens. *In* M. C. Appleby, R. I. Horrell, J. C. Petherick, and S. M. Rutter (Eds.), *Applied animal behaviour: past present and future*, pp. 132-133. UFAW, Potters Bar, Herts.

Van Liere, D. W., and P. R. Wiepkema. 1992. Effects of long-term deprivation of sand on dustbathing behaviour in laying hens. Anim. Behav. 43: 549-558.

Vestergaard, K., and J. A. Hogan. 1992. The development of a behavior system: dustbathing in the burmese red junglefowl. III effects of experience on stimulus preference. Behaviour 121: 215-230.

Vestergaard, K. S. 1982. Dust-bathing in the domestic fowl - diurnal rhythm and dust deprivation. Appl. Anim. Ethol. 8: 487-495.

Vestergaard, K. S., J. A. Hogan, and J. P. Kruijt. 1990. The development of a behavior system: dustbathing in the burmese red junglefowl 1. The influence of the rearing environment on the organization of dustbathing. Behaviour 112: 99-116.

Vestergaard, K. S., E. Skadhauge, and L. G. Lawson. 1997. The stress of not being able to perform dustbathing in laying hens. Physiol. Behav. 62: 413-419.

Wechsler, B., and B. Huber-Eicher. 1998. The effect of foraging material and perch height on feather pecking and feather damage in laying hens. Appl. Anim. Behav. Sci. 58: 131-141.

Widowski, T. M., and I. J. H. Duncan. 2000. Working for a dustbath: are hens increasing pleasure rather than reducing suffering? Appl. Anim. Behav. Sci. 68: 39-53.

Annex 1.

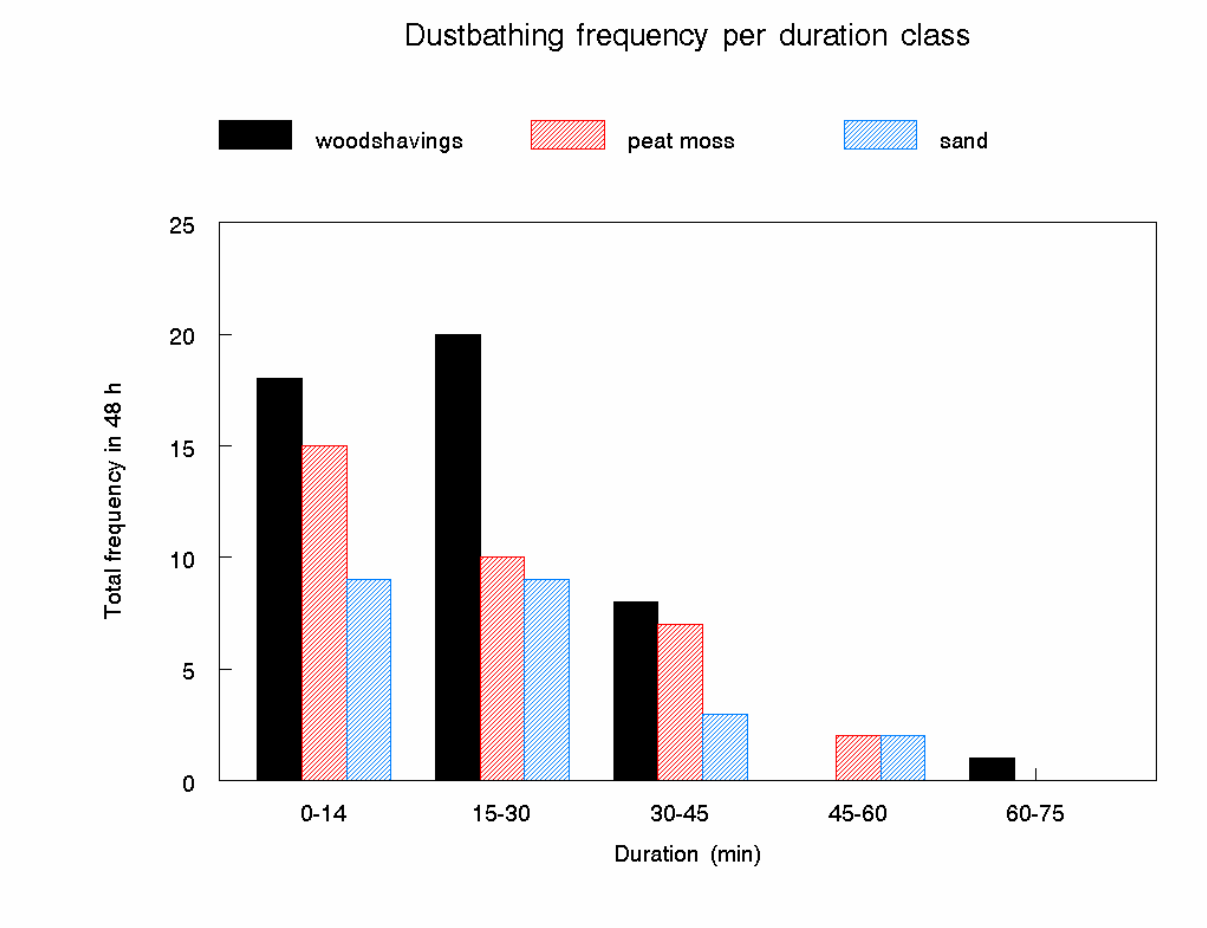

Figure A. Total frequency of dustbathing in 48 hours per substrate type categorised in different duration classes.

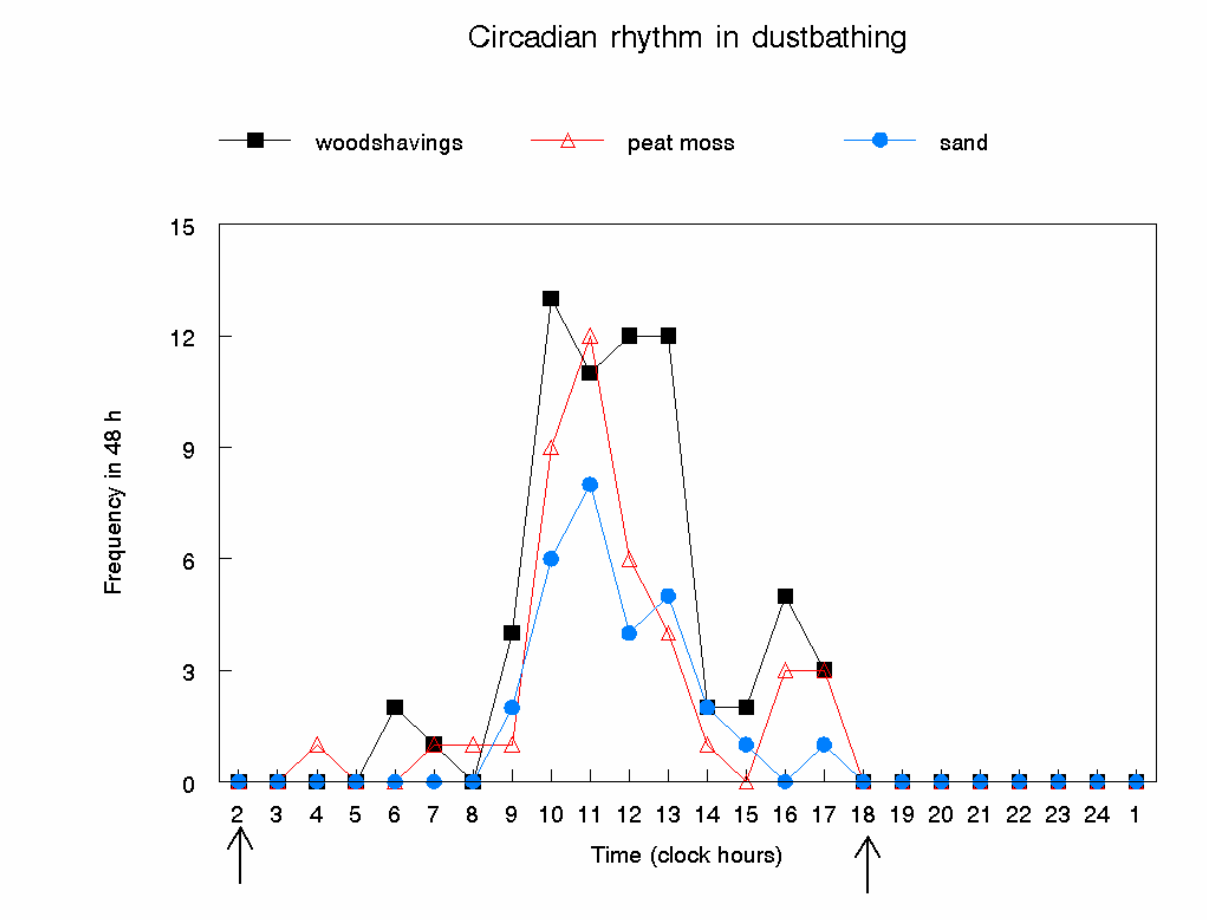

Figure B. Rhythm in dustbathing behaviour over the light period. The Y-axis shows the total frequency of dustbathing per 48 h of observation.