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FACHBEREICH ÖKOLOGISCHE AGRARWISSENSCHAFTEN

**Exploratory and dust-bathing behaviour in
laying hens kept in commercial aviaries and
furnished cages**

Dissertation zur Erlangung des akademischen Grades eines Doktors der
Agrarwissenschaften (Dr. agr.)

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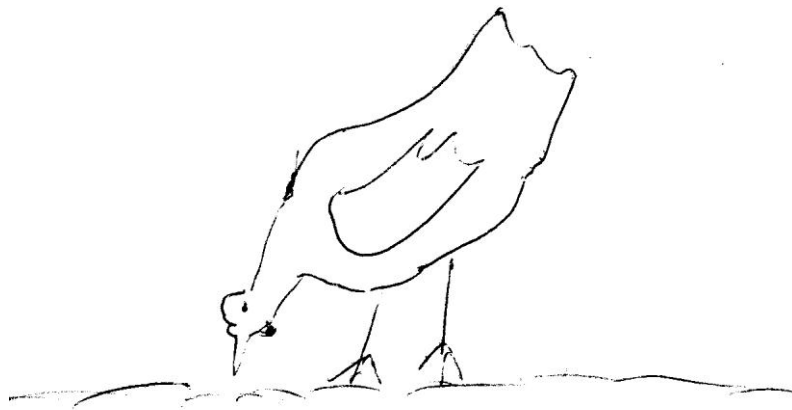
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Dedicated to my parents



Contents

List of figures	iv
List of tables	vi
1. General introduction	1
1.1 Literatur	2
2. How to observe exploratory and dust-bathing behaviour in commercial laying hens kept in aviaries? – An on-farm pilot study	4
2.1 Introduction	4
2.2 Animals, materials and methods	5
2.2.1 Aviary systems and flocks	5
2.2.2 Video recordings	7
2.2.3 Direct and video observations	11
2.2.4 Analysis of behavioural data	14
2.3 Results	14
2.3.1 Explorative data of direct observations and video analysis	14
2.3.1.1 Direct observations	14
2.3.1.2 Video analysis	15
2.3.2 Determination of sample interval	16
2.3.3 Observation time	16
2.3.4 Intra- and inter-observer reliability	17
2.3.4.1 Intra-observer reliability	17
2.3.4.2 Inter-observer reliability	17
2.4 Discussion	18
2.4.1 Direct observations in commercial flocks	18
2.4.2 Video observations in commercial flocks	20
2.4.3 Determination of sample interval	21
2.4.4 Observation time	21
2.4.5 Intra- and inter-observer reliability	22
2.4.5.1 Intra-observer reliability	22
2.4.5.2 Inter-observer reliability	22
2.5 Conclusion	23
2.6 Literature	23
3. Exploratory and dust-bathing behaviour in laying hens kept in aviary systems – identification of influencing factors	27

3.1 Introduction	27
3.2 Animals, materials and methods	29
3.2.1 Aviary systems and flocks	29
3.2.2 Video recordings and behavioural observations	31
3.2.3 Further measures	33
3.2.4 Intra- and interobserver reliability and statistical analysis	34
3.3 Results	34
3.3.1 Intra- and inter-observer reliability	34
3.3.2 Farm characteristics	35
3.3.3 Data overview and analysis of variance	37
3.4 Discussion	41
3.4.1 Extent of exploratory and dust-bathing behaviour in the litter area	41
3.4.2 Litter height	42
3.4.3 Litter type and quality	43
3.4.4 Litter humidity	45
3.4.5 Stocking density and group size	45
3.4.6 Light intensity	46
3.5 Conclusion	46
3.6 Literature	47
4. Exploratory and dust-bathing behaviour in laying hens kept in furnished cages – identification of influencing factors	52
4.1 Introduction	52
4.2 Animals, materials and methods	53
4.2.1 Housing systems and flocks	53
4.2.2 Video recordings and behavioural observations	55
4.2.3 Further measures	58
4.2.4 Intra- and inter-observer reliability and statistical analysis	60
4.3 Results	60
4.3.1 Intra- and inter-observer reliability	60
4.3.2 Farm characteristics	61
4.3.3 Data overview and analyses of variance	62
4.4 Discussion	65
4.4.1 Behaviours	65
4.4.2 Substrate availability	66

4.4.3 Number of mats per cage, mat width and mat area per hen	67
4.4.4 Cage stocking density and group size	68
4.4.5 Light intensity	69
4.5 Conclusion	70
4.6 Literature	70
5. The influence of litter management on the qualitative performance of dust-bathing behaviour in laying hens kept in aviary systems	74
5.1 Introduction	74
5.2 Animals, materials and methods	75
5.2.1 Video recordings	75
5.2.2 Behavioural observations	76
5.2.3 Statistical analysis	77
5.3 Results	78
5.3.1 Inter-observer reliability	78
5.3.2 Overview over data	78
5.3.3 Analyses of variance	82
5.4 Discussion	85
5.4.1 Dust-bathing parameters	85
5.4.1.1 Total dust-bath duration	85
5.4.1.2 Proportion of the tossing phase, absolute number of vertical wing shaking and scratching with one leg per dust-bath and frequency per minute tossing phase	86
5.4.2 Influencing factors	87
5.4.2.1 Litter height	87
5.4.2.2 Litter type	87
5.4.2.3 Disturbance class	89
5.4.2.4 Light intensity	89
5.4.2.5 Stocking density	90
5.5 Conclusion	90
5.6 Literature	91
6. General conclusions	95
7. Summary	96
8. Zusammenfassung	99

List of figures

Figure 2.1: NATURA-Nova 250 aviary system (Big Dutchman 2012, own captions)	6
Figure 2.2: Bolegg Terrace aviary system (Vencomatic 2012, own captions)	6
Figure 2.3: Examples of camera positioning indoors of the NATURA-Nova system	8
Figure 2.4: Examples of camera positioning in the winter garden of the NATURA-Nova system	9
Figure 2.5: Examples of camera positioning of the Bolegg Terrace system	10
Figure 2.6: Sketch of the NATURA-Nova aviary with sizes of the indoor litter area and winter garden with corresponding parts and their sizes for direct observations	12
Figure 2.7: Sketch of the Bolegg Terrace aviary with sizes of the litter area with corresponding parts and their sizes for direct observation	12
Figure 2.8: Frame on a video picture opened with the Observer XT 10.5	13
Figure 3.1: Example of portal-system: NATURA-Nova Twin aviary portal-system with winter garden to both sides (Big Dutchman 2012, own captions)	30
Figure 3.2: Screenshot of the observation frame (1 sqm) on a video picture opened with the Observer XT 10.5 (bodies of three hens outside the frame are blurry because hens were moving)	33
Figure 3.3: Distribution of observation areas in terms of litter humidity and the three litter categories (1 = missing litter, 2 = fine material, 3 = fine material mixed with straw or pure straw)	36
Figure 3.4: Distribution of observation areas in terms of litter quality (identifiable or not identifiable) and two litter categories (2 = fine material, 3 = fine material mixed with straw or pure straw)	37
Figure 3.5: Total number of hens in the single observation areas (n = 81) distributed to the 22 farms	38
Figure 3.6: Percentages of hens pecking, scratching and dust-bathing in the single observation areas (n = 81) distributed to the 22 farms	38
Figure 3.7: Percentages of hens scratching in dry or humid litter Different letters denote significant differences, $p = 0.05$ (least squares means of analysis of variance with farm set as random factor, $n = 81$, $df = 72$)	40

Figure 3.8: Percentages of hens dust-bathing in different litter types (1 = missing litter, 2 = fine material, 3 = fine material and straw mixed or pure straw) Different letters denote significant differences, $p = 0.05$ (least squares means of analysis of variance with farm set as random factor, $n = 81$, $df = 72$)	40
Figure 4.1: Farmer Automatic Maxi System (Farmer Automatic 2012, own captions)	54
Figure 4.2: Furnished cage system from Zucami with 8 floors (own picture)	55
Figure 4.3: Position of camera directed at scratching mat in the furnished cage system KV 1500 from Big Dutchman (own picture)	56
Figure 4.4: Upper picture: Mat with unperforated flat surface; middle picture: perforated flat surface; lowest picture: synthetic turf (own pictures)	59
Figure 4.5: Percentages of hens on total mat area, and percentages of hens pecking, scratching and dust-bathing on the observed scratching mats ($n = 57$) distributed to the 16 farms	63
Figure 5.1: Distribution of analysed dust-bathing events (129 in total) in terms of litter height in the respective litter areas	79
Figure 5.2: Distribution of analysed dust-bathing events (129 in total) with regard to the four litter material classes on which they were performed, number of farms in brackets	80
Figure 5.3: Frequency classes concerning disturbances per dust-bath	80
Figure 5.4: Distribution of analysed dust-bathing events (129 in total) with regard to the average light intensity in the litter areas	81
Figure 5.5: Distribution of total dust-bath durations (129 dust-bathing events)	81
Figure 5.6: Correlation between vertical wing shaking and scratching with one leg, based on 129 dust-bathing events observed	82
Figure 5.7: Dust-bath duration, proportion of the tossing phase, absolute number of vertical wing shaking per dust-bath and frequency per minute tossing phase corresponding to the four litter type classes (1 = missing litter, 2 = fine materials, 3 = fine materials mixed with straw, 4 = straw; least squares means, standard deviations and p-values of the significant results (analyses of variance, $n = 129$, $df = 118$), significance level: $p = 0.05$)	84
Figure 5.8: Dust-bath duration and frequency of vertical wing shaking per minute tossing phase corresponding to the three dust-bath disturbance classes; least squares means, standard deviations and p-values of the significant results (analyses of variance, $n = 129$, $df = 118$), significance level: $p = 0.05$)	85

List of tables

Table 2.1: Overview over video and direct recordings	7
Table 2.2: Definitions of the assessed parameters	13
Table 2.3: Mean, standard deviation, minimum and maximum of percentages of hens performing vertical wing shaking and axial body shaking, direct observations of indoor litter area (n = 3) and winter garden (n = 4) of flock 1 and of flock 2 (n = 9)	15
Table 2.4: Mean, standard deviation, minimum and maximum of total numbers of hens and percentages of hens performing scratching & pecking and vertical wing shaking, video observations of flock 1 (n = 12) and 2 (n = 8)	15
Table 2.5: Spearmans` rank correlation coefficients for total numbers of hens and percentages of hens scratching & pecking and dust-bathing calculated on the basis of one minute sampling intervals in relation to two, three, five and ten minutes (n = 8)	16
Table 2.6: Spearmans` rank correlation coefficients for total numbers of hens and percentages of hens scratching & pecking and vertical wing shaking on the basis of one hour observation time in relation to 16 and 30 minutes (n = 8)	16
Table 2.7: Intra-observer reliability (Spearmans` rank correlation coefficients) concerning total number of hens and non-evaluable hens and percentages of hens scratching & pecking, dust-bathing, searching and preening during 16 minutes observations with a sampling interval of 2 minutes for two observation sessions (n = 17)	17
Table 2.8: Inter-observer reliability (Spearmans` rank correlation coefficients) concerning total number of hens and non-evaluable hens and percentages of hens scratching & pecking, dust-bathing, and preening during 16 minutes observations with a sampling interval of 2 minutes recorded by two observers (n = 17)	17
Table 3.1: Regional distribution of aviaries included in the study	29
Table 3.2: Distribution of types of aviaries included in the study	30
Table 3.3: Definitions of the assessed parameters in aviaries	32
Table 3.4: Scoring of litter humidity after trying to form a ball from the litter	33
Table 3.5: Pearson correlation coefficients for intra-observer (n = 15) and inter-observer reliability (n = 13) concerning total number of hens and percentages of hens non-evaluable, pecking, scratching and dust-bathing	34
Table 3.6: Characteristics of the 22 visited flocks in terms of flock and group size, number of pens per aviary, stocking density in the observed pen and litter area, size of litter area per pen, litter height and light intensity	35

Table 3.7: Distribution of observation areas (n=81) and aviaries in terms of categories of litter material used	36
Table 3.8: Results of analysis of variance concerning influencing factors on total number of hens, percentages of hens pecking, scratching and dust-bathing with farm set as random factor (n = 81, df = 72)	39
Table 4.1: Regional distribution of furnished cages included in the study	54
Table 4.2: Distribution of types of furnished cages included in the study	54
Table 4.3: Definitions of the assessed parameters in furnished cages	57
Table 4.4: Intra- and inter-observer reliability (Spearman's` rank correlation coefficients) for total number of hens and percentages of hens non-evaluable, pecking, scratching and dust-bathing (n = 14 cameras)	61
Table 4.5: Characteristics of the 16 visited flocks in terms of flock, group and cage size, cage number per flock, stocking density and scratching mat number, length, width, total area, area of one mat and area per hen, as well as light intensity in the scratching area	61
Table 4.6: Results of analyses of variance concerning influencing factors on percentages of hens on total mat area, pecking, scratching and dust-bathing with farm set as random factor (n = 57, df = 48)	64
Table 5.1: Definitions of the recorded dust-bathing elements	77
Table 5.2: Definitions of the recorded dust-bathing phases	77
Table 5.3: Inter-observer reliability between two observers (n=16)	78
Table 5.4: Influencing factors on total dust-bath duration, proportion of the tossing phase, total number of vertical wing shaking per dust-bath and frequency per minute tossing phase (analyses of variance with farm and camera set as random factors, n = 129, df = 118)	83

1. General introduction

In the year 2011 in Germany 34 million laying hens were kept in 1,224 farms; housing capacity was at even 40 million hen places. After a decreased egg production as a result of legal amendments in the years 2008 and 2009, the laying hen sector in Germany noted a plus of about 14 % concerning hen numbers in 2011 compared to 2010. Of the birds 63 % were housed in floor systems, each 15 % in furnished cages ('small group cages') or free-range systems and 7 % in organic production (DESTATIS 2012). Conventional cages were replaced by furnished cages or non-cage systems in Germany as from 2010 at the latest (TierSchNutzV 2006) and are banned EU-wide as from the beginning of the current year 2012 (EC Directive 1986). Currently the German legal standards on furnished cages are suspended due to a decision of the Federal Constitutional Court for formal reasons, but the material requirements are still applied by the competent authorities. It is debated whether also the use of furnished cages shall be banned, and if so, how long a transition period should last, but a decision and change of the welfare regulation is currently not foreseeable.

Within the non-cage systems, aviaries are increasingly used. They have the advantage that the housing capacity per unit floor area is increased by use of the third dimension. Both, the furnished cages and aviaries provide more or less spatially divided functional areas for the different species-specific behaviours. The central resources in these areas are the feeders, drinkers, nest boxes, perches and littered areas. Furnished cages provide more limited space and thus freedom for movement. While in floor systems according to the welfare regulation (TierSchNutzV 2006) at least 250 sqcm litter area per hen must be provided, in the cages it need only be 90 sqcm. Litter is especially important for laying hens to stimulate and enable their exploratory and dust-bathing behaviour. For the laying hens' ancestors exploration of their surrounding as well as 'information gathering' was determining for their survival in the wild (EFSA 2005). The foraging behaviour, as an important element of exploration, includes besides walking, stepping back and manipulating with the bill above all the pecking and scratching behaviour. Exploratory (foraging) behaviour constitutes the most time-consuming behaviour in domestic chickens, even under artificial conditions as in laying hen houses (EFSA 2005). Further, the dust-bathing behaviour as one of the most complex behaviour in domestic fowl (Fölsch 1981) is performed especially under dry and warm conditions on loose soil (Krujic 1964). In the hen houses litter should provide an adequate alternative to the loose, dry soil the hens' wild conspecifics find in nature. Besides enabling hens to perform their species-specific behaviours, litter further allows the hens to keep their bill and claws in a physiological state, thereby reducing the injurious potential in case of pecking at conspecifics and the risk of broken claws as well as the associated pain (Fölsch et al. 1986). Moreover, forage enrichments are the most effective device to reduce feather pecking (Nørgaard-Nielsen 1997, Dixon et al. 2010), but it is necessary to provide adequate litter already from hatching to lower the risk of feather pecking and cannibalism (Johnsen et al. 1998). Though litter fulfils valuable functions for hens, some manipulative behavioural elements with the bill such as mincing or tearing as carried out at natural feed plants are less stimulated or not possible (Martin 1985).

Both, the exploratory and dust-bathing behaviour are well examined under experimental conditions. In contrast, up to date on-farm studies in furnished cage and aviary systems on

these topics are largely lacking. Especially there is a need for scientific insights into the welfare aspects of both housing systems regarding animal behaviour. This lack of published work is certainly partly due to the methodical challenges that are associated with behavioural observations in commercial animal houses, especially regarding large groups composed of small animals (Knierim and Winckler 2009).

In the following chapters a possible approach to the study of behaviour on-farm is presented and discussed. Using this approach, analyses are undertaken in order to identify important housing and management factors that affect the extent and detailed expression of exploratory and dust-bathing behaviour in different types of furnished cages and aviary systems under commercial conditions. Finally, based on the results, it shall be considered which management measures can be recommended for laying hen farmers in order to enhance hen welfare or in which areas further research is needed.

These studies have been part of a large cooperative research project for the development of recommendations for the on-farm management of furnished cages for laying hens in comparison with aviaries. The project was founded by the Ministry of food agriculture and consumer protection (BMELV) by the agency of the Federal Institution of agriculture and food (BLE).

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2. How to observe exploratory and dust-bathing behaviour in commercial laying hens kept in aviaries? – An on-farm pilot study

2.1 Introduction

There has been a substantial change within recent years in keeping laying hens in Germany, back from extremely barren, standardized production systems to systems which provide environmental enrichment in order to better fulfill animals' needs and to enhance their welfare. The development of aviaries, beginning in Great Britain and Switzerland since the seventies, implied different functional areas like nest boxes and litter areas within the hen house that enable laying hens to exercise maintenance behaviours, although this was rather a side effect because the aim was to increase stocking density by implementing platforms (Fölsch 1983, Bessei 1997, Cooper and Albentosa 2003). According to the German welfare regulation Tierschutz-Nutztierhaltungsverordnung (TierSchNutzV 2006) a litter area with suitable, friable substrate in sufficient amounts must be provided for laying hens kept in aviaries. Every hen should be able to satisfy their behavioural needs such as pecking, scratching and dust-bathing. At least one third of the accessible floor area has to be littered and available unrestrictedly daily for at least two thirds of the lighting hours. It is possible to provide the litter area as a winter garden (TierSchNutzV 2006). Exploratory and foraging as well as dust-bathing behaviours belong to the laying hens' natural behavioural repertoire and the possibility to express natural behaviours can increase welfare (Botreau et al. 2007). There is evidence that a lack of possibility to perform dust-bathing leads to stress (Vestergaard et al. 1997), whereas if opportunity is provided, on average they dust-bath every second day for about 20 - 35 minutes (Fölsch 1981, Vestergaard 1982, Van Liere et al. 1990). Compared to conventional cages, thus, hens' welfare can be enhanced in aviaries concerning dust-bathing behaviour (Colson et al. 2007).

Further, research under near-natural conditions in a relatively small group of semi-wild Red Junglefowl showed that 60 % of the active part of a day is used for ground pecking and 34 % for ground scratching (Dawkins 1989). Foraging behaviours still exist in domestic chicken, whereas the extent varies between strains of hybrids. Nevertheless, laying hens spend more than 30 % of their time a day on feeding and foraging (reviewed by EFSA 2005). They scratch intensively on the ground and search for food, particles are manipulated by the bill and pecked. The latter behaviour can occur up to 15,000 times per day (Savory et al. 1978, Picard et al. 2002, Webster 2002). Furthermore, laying hens show a preference for additional foraging material in choice tests (Nicol et al. 2009), and the time they spent on exploratory and manipulative foraging behaviour is inversely related to the rate of feather pecking, which is commonly seen as redirected foraging behaviour (Huber-Eicher and Wechsler 1998, Aerni et al. 2000, Keeling 2002, EFSA 2005). Consequently, Dixon et al. (2010) recommend forage enrichments for poultry housings as the most effective device to reduce feather pecking. In addition, Johnsen et al. (1998) found that it is important to provide adequate material already after hatching.

In the light of the undeniable importance and the current legislation concerning the provision of adequate litter for laying hens, more information is needed about possible effects of the management of the litter area on the exploratory and dust-bathing behaviour of laying hens in

commercial aviary systems. The current pilot study should help to work out methodical approaches to analyse these behaviours on-farm. Behavioural small-scale observations of exploratory and dust-bathing behaviour have been carried both live (directly, e.g. Vestergaard 1982, Blokhuis 1984, Petherick and Duncan 1989, Vestergaard et al. 1990, Bubier 1996) and from videos (e.g. Van Liere and Bokma 1987, Van Liere et al. 1990, Van Liere and Wiepkema 1992, Colson 2007, Moesta et al. 2008). However, on-farm behavioural observations in large groups and complex housing systems are methodologically challenging. Direct observations have limitations in terms of observation times and also possible disturbances of the laying hens by the observer. Video observations may pose difficulties in terms of identification of behaviour and only parts of the hen houses can be surveyed due to economic limitations regarding number of observation cameras and observation time of video recordings. Thus, the focus of the pilot study was to determine adequate sample intervals, observation times and numbers of observation hours per day. For behaviours taking place at specific places in the house which cannot completely be predicted (dust-bathing), it was a further aim to assess the suitability of video versus direct observations. Finally, intra- and inter-observer reliabilities were tested regarding video observations, applying the selected observation methods.

2.2 Animals, materials and methods

2.2.1 Aviary systems and flocks

The study was conducted in spring 2010 at a large German commercial laying hen farm in two flocks, one with Lohmann Brown hens (flock 1) aged 27 weeks, and one with LSL hens (flock 2) aged 66 weeks, with flock sizes and stocking densities of 6,000 and > 18 hens/sqm (area under the first platform and the right litter area part were not accessible at first weeks of the laying period to prevent hens lay their eggs outside the nest boxes, which increased the stocking density to over 18 hens/sqm) as well as 8,000 and 14 hens/sqm. Flock 1 was housed in a NATURA-Nova aviary system (Big Dutchman, Figure 2.1 shows a system with two rows, the studied aviary just had one), divided in two pens and with a winter garden. Flock 2 was housed in a Bolegg Terrace aviary system (Vencomatic, Figure 2.2 shows a system with three rows, the studied aviary just had two) without winter garden that was divided in four pens. In both flocks litter material was sand at a height of four centimetres at maximum in flock 1 and one centimetre in flock 2, the winter garden had no litter.

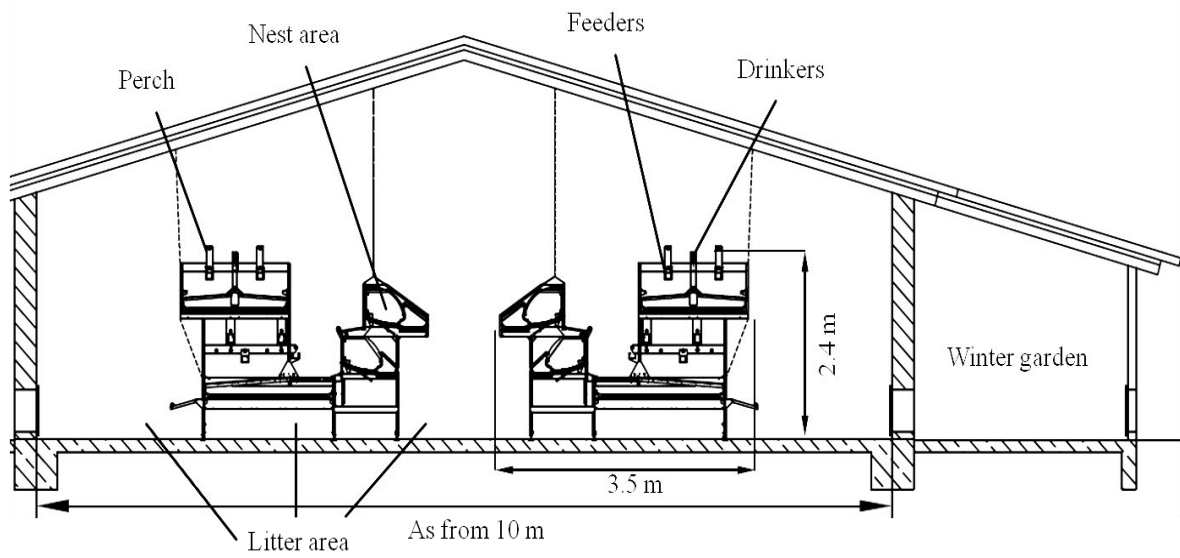


Figure 2.1: NATURA-Nova 250 aviary system (Big Dutchman 2012, own captions)

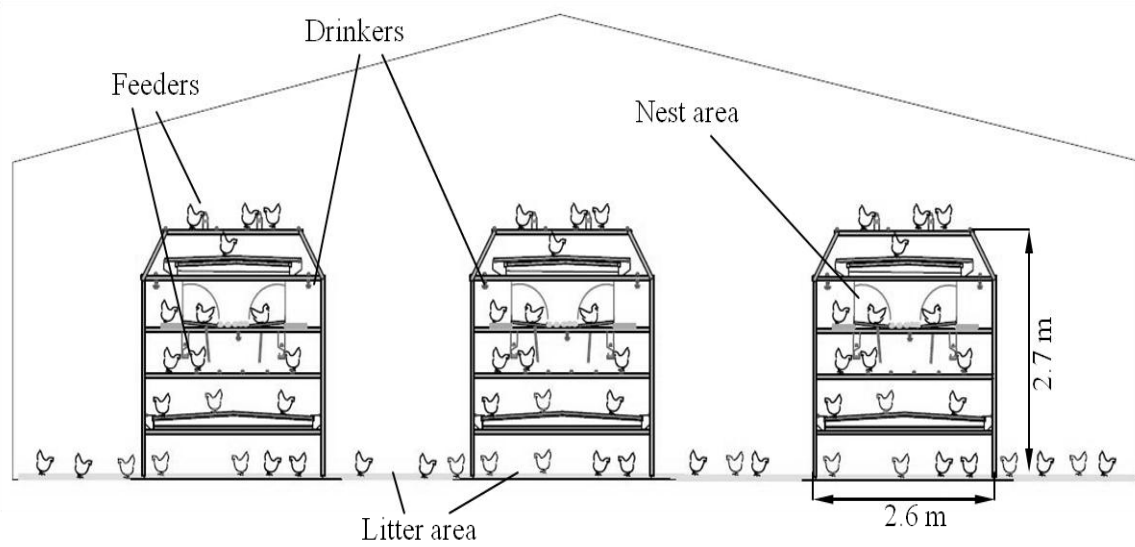


Figure 2.2: Bolegg Terrace aviary system (Vencomatic 2012, own captions)

2.2.2 Video recordings

Altogether 15 cameras were used in each system: Two Axis 221 Network Cameras (640x480 pixels, 24VAC/DC) with night and day function, vario lenses (120°) and dust cover were installed in the NATURA-Nova system, one outdoors and one indoors. All other cameras in both systems were SANTEC colour cameras for corner mount (VTC-E220IRP) with IR-LEDs, 550 TVL and 4.3 mm lenses. Four SANTEC cameras each were connected to one self-composed server. Videos were saved on external hard drives (One Technologies, 1.5 TB). The videos from the Axis cameras were recorded and edited by the Axis Camera Station 3.0 Software on another server (Fujitsu Primergy TX150s6). Axis cameras were not used in the Bolegg Terrace system again because of less easy handling due to greater size and weight.

The cameras were distributed over the litter areas of the first pen of each aviary as evenly as possible (examples of the camera positioning see Figures 2.3 to 2.5). One day before the recording period started, in both flocks cameras were fixed with wall arms and laces on walls or structural elements, each at about the same height. Cameras which filmed under the system in flock 2 could not be analysed reliably due to hens blocking the view or reflections by relatively bright light. Furthermore, malfunctions of the camera equipment in both flocks led to a lower number of observable cameras than 15 per flock (Table 2.1).

Recording ran over three days (72 hours), from which the first recorded day was not analysed because hens should have enough time to adapt to the equipment. Only the videos from the following day were used during the lighting hours for behavioural observations. At the third day direct observation took place concurrently (Table 2.1).

Table 2.1: Overview over video and direct recordings

	Flock 1 (NATURA-Nova system)	Flock 2 (Bolegg Terrace system)
Indoor lighting times/ opening times of winter garden	Indoors: 05:00 - 21:30 h Winter garden: 10:00 - 22:30 h	04:00 - 21:00 h
Video observations:		
Date	9 - 10 May 2010	19 - 20 May 2010
Number of cameras installed and usable for behavioural observations (in brackets)	Indoors: 8 (6) Winter garden: 7 (6)	15 (8): 9 (8) upper cameras; 6 (0) cameras under the system
Observation time	Indoors: 13:00 - 14:00 h Winter garden: 20:00 - 21:00 h	06:00 - 07:00, 13:00 - 14:00, 16:00 - 17:00 h
Direct observations:		
Date	10 May 2010	20 May 2010
Observation time	Indoors: 09:30 - 12:30 h Winter garden: 13:30 - 15:30 h	Mornings: 09:50 - 11:30 h Afternoons: 12:20 - 14:00 h

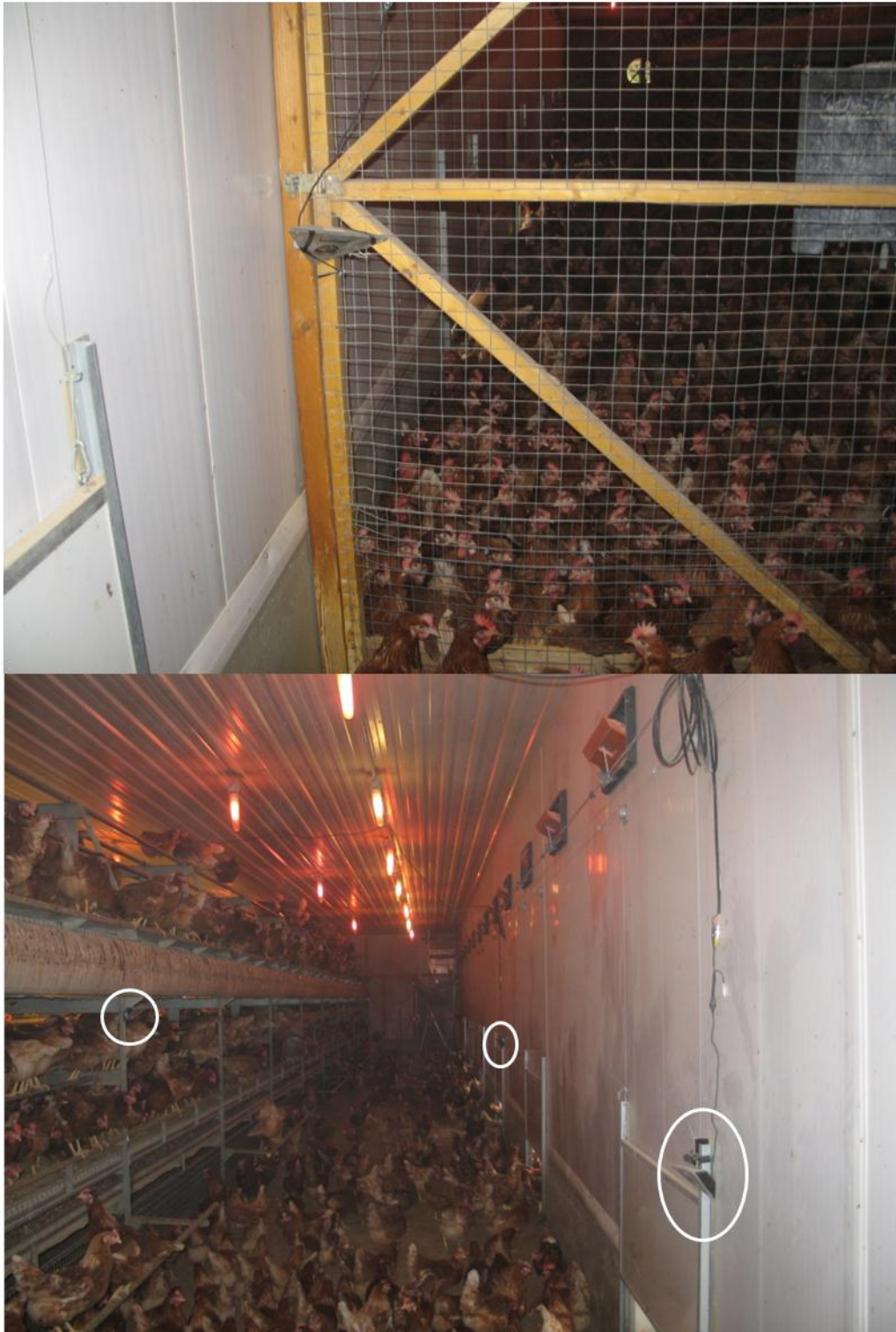


Figure 2.3: Examples of camera positioning indoors of the NATURA-Nova system



Figure 2.4: Examples of camera positioning in the winter garden of the NATURA-Nova system



Figure 2.5: Examples of camera positioning of the Bolegg Terrace system

2.2.3 Direct and video observations

Direct observations were carried out implementing the instantaneous scan sampling method (Martin and Bateson 2007) with a sample interval of one minute by use of a stopwatch. Data were entered by hand into the data sheets.

Regarding the NATURA-Nova system indoor litter area and winter garden were divided into three and four parts of equal size, respectively (Figure 2.6). During the observations the observer sat on a box at the following positions:

Indoors:

- Between parts 1 and 2 (observing both parts successively for each 60 minutes)
- Between parts 2 and 3 (observing only part 3 for 60 minutes)

Winter garden:

- Between parts 1 and 2 (observing both parts successively for each 30 minutes)
- Between parts 3 and 4 (observing both parts successively for each 30 minutes)

Both, in- and outdoors, hens had 15 minutes adaptation time to the observer before observations were started.

In flock 2 the directly observed parts corresponded to the upper nine camera positions (Figure 2.7). Positions filmed under the system could not be directly observed. The observer stood close to the system and observations were carried out for 10 minutes per position. Before beginning the first observation and always after walking to the next litter area (left, middle and right) hens had five minutes adaptation time to the observer before observations started. All positions were observed twice in the morning and afternoon (Table 2.1).

Video analysis was done with the Observer XT 10.5 (Noldus Information Technology, Wageningen, The Netherlands). The video picture size was always 17 cm in length and 13 cm in height. Because this picture was too large for a consistently reliable recording, a frame made out of pasteboard with 8 cm length and 6 cm height was always put on the same position on the screen picture. The area within the frame was then observed (Figure 2.8).

Video observations were carried out using instantaneous scan sampling (Martin and Bateson 2007) with a sample interval of one minute. Always the first five seconds of every sample point, or further five seconds regarding dust-bathing and preening if behaviour was not clearly identifiable, were observed. Pecking and scratching had to be identified within the first five seconds.

Number of hens within the frame as well as their exploratory, dust-bathing or preening behaviour and the number of hens with non-evaluable behaviour were recorded as described in Table 2.2. The percentages of hens showing a certain behaviour were calculated with reference to the number of hens evaluable.

Winter garden Size: 97 sqm	Litter area left Size: 66 sqm	Aviary-row	Litter area right
Part 1 24 sqm	Part 1 22 sqm		Not accessible for hens
Part 2 24 sqm	Part 2 22 sqm		
Part 3 24 sqm	Part 3 22 sqm		
Part 4 24 sqm	Part 3 22 sqm		

Figure 2.6: Sketch of the NATURA-Nova aviary with sizes of the indoor litter area and winter garden with corresponding parts and their sizes for direct observations

Litter area left Size: 21 sqm	Aviary-row left	Litter area middle Size: 36 sqm	Aviary-row right	Litter area right Size: 21 sqm
Part 1 7 sqm		Part 4 12 sqm		Part 7 7 sqm
Part 2 7 sqm		Part 5 12sqm		Part 8 7 sqm
Part 3 7 sqm		Part 6 12 sqm		Part 9 7 sqm

Figure 2.7: Sketch of the Bolegg Terrace aviary with sizes of the litter area with corresponding parts and their sizes for direct observation

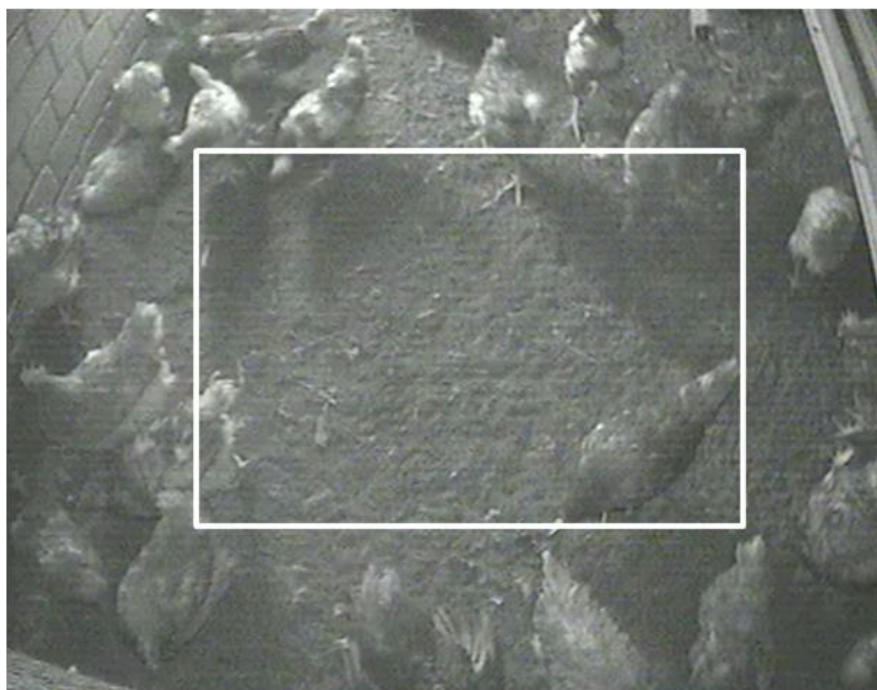


Figure 2.8: Frame on a video picture opened with the Observer XT 10.5

Table 2.2: Definitions of the assessed behavioural parameters

Total number of hens	Hens partly (even if only with bill, foot or other body part) or completely visible within the observation area (frame)
Exploratory behaviour:	
Scratching	Number of hens performing fast movements with one or alternately both foot/feet on the ground of the litter area
Pecking	Number of hens performing a short, fast movement with head towards the ground
Scratching and pecking	The calculated sum of hens scratching and pecking
Searching	Number of hens holding head near to ground of the litter area while walking
Dust-bathing behaviour	Number of hens showing <u>vertical wing shaking</u> (lying on their breast with fluffed feathers and showing fast vertical wing movements, immediately followed by rhythmic movements of the legs; Severin 2002, modified after Van Liere and Wiepkema 1992) or –in direct observations- <u>axial body shaking</u> (standing and shaking their bodies along the axis of the trunk with fluffed feathers and somewhat lifted wings in short and becoming faster movements (modified after Moesta et al. 2008, Fölsch 1981)
Preening	Number of hens cleaning their plumage with the bill or scratching their head with one leg while standing or lying
Total number of hens non-evaluable	Number of hens whose behaviour cannot be identified doubtlessly; for the calculation of the average percentages of hens performing certain behaviours at any time, these hens are subtracted from the total number of observed hens.

2.2.4 Analysis of behavioural data

For direct observations, mean, standard deviation, minimum and maximum of total number of hens observed as well as percentages of hens performing vertical wing shaking and axial body shaking were calculated.

From the video data, the average number of hens observed and non-evaluable as well as the percentages of observed time the average hen performed scratching, pecking, searching, preening or vertical wing shaking (corresponding to the average percentage of hens performing a behaviour at any time) were calculated, summing up all sample points per camera. To address the question of adequate sample intervals, videos from two light hours (starting at 06:00 and 16:00 hours) and eight cameras in flock 2 were used to simulate sample intervals of two, three, five and ten minutes by taking every second, third, fifth or tenth sample point. Spearman's rank correlation coefficients were calculated between the different results. As a limit for adequate correlation $r_{\text{Spearman}} \geq 0.7$ was set. The longest sample interval that showed sufficient correlation with the results from 1 minute instantaneous scan sampling was used further to test for adequate observation times. The average number of hens and percentages of time performing the different behaviours were calculated either based on the whole dataset, or only the first 30 minutes per hour or 16 minutes per hour. Again the limit of $r_{\text{Spearman}} \geq 0.7$ was used to decide for an appropriate observation time.

Finally, for testing of intra- and inter-observer reliability between two observers, the data from altogether 17 cameras were used, covering one hour from 13:00 to 14:00 hours (six indoor cameras in flocks 1, five in flock 2) and from 20:00 to 21:00 hours (six cameras in the winter garden in flock 1) As a limit for an adequate correlation a $r_{\text{Spearman}} \geq 0.7$ was set (Martin and Bateson 2007).

The Spearman's rank correlations were calculated in SPSS 19.0.0.1 (SPSS, IBM Company, Illinois), all other parameters with Excel (Microsoft Office Excel 2007).

2.3 Results

2.3.1 Explorative data of direct observations and video analysis

2.3.1.1 Direct observations

The total number of hens within the observed litter area of flock 1 could only be estimated: On average it were about 215 ± 76 (min - max: 80 - 400) hens on 22 sqm large areas indoors and 231 ± 121 (min - max: 50 - 400) on 24 sqm large areas in the winter garden. In flock 2 on average 15 ± 7 (min - max: 1 - 30) hens were counted on areas of 7 to 12 sqm.

The percentage of observed time the average hen performed dust-bathing behaviour was low in both flocks, between on average 0 and 0.5 % (Table 2.3).

Table 2.3: Mean, standard deviation, minimum and maximum of percentages of hens performing vertical wing shaking and axial body shaking, direct observations of indoor litter area (n = 3) and winter garden (n = 4) of flock 1 and of flock 2 (n = 9)

	% of hens vertical wing shaking	% of hens axial body shaking
Flock 1, indoor litter area 09:30 – 12:30 hour		
Mean, standard deviation	0.5 ± 0.7	0.1 ± 0.3
Min	0	0
Max	3.3	2
Flock 1, winter garden 13:30 – 15:30 hour		
Mean, standard deviation	0.1 ± 0.3	0 ± 0.2
Min	0	0
Max	1.5	1.3
Flock 2 09:50 – 11:30, 12:20 – 14:00 hour		
Mean, standard deviation	0.3 ± 1.4	0.2 ± 1.1
Min	0	0
Max	10.5	8.3

2.3.1.2 Video analysis

Within the observation frame on average two to 29 hens were counted in both flocks. Percentages of hens performing scratching and pecking behaviour were between 0 and 22 %, for dust-bathing behaviour the range was from 0 to 16 % (Table 2.4).

Table 2.4: Mean, standard deviation, minimum and maximum of total numbers of hens and percentages of hens performing scratching & pecking and vertical wing shaking, video observations of flock 1 (n = 12) and 2 (n = 8)

	Total numbers of hens	% of hens scratching & pecking	% of hens vertical wing shaking
Flock 1, indoor litter area 13:00 hour			
Mean, standard deviation	10.6 ± 5.4	6.8 ± 6.8	5.9 ± 6.1
Min	3.5	0.0	0.0
Max	18.3	18.4	15.7
Flock 1, winter garden 20:00 hour			
Mean, standard deviation	17.2 ± 6.6	10.7 ± 5.6	1.2 ± 1.6
Min	11.5	2.8	0.0
Max	29.0	20.0	3.9
Flock 2 06:00, 13:00, 16:00 hour			
Mean, standard deviation	7.2 ± 4.7	11.7 ± 5.6	0.0 ± 0.1
Min	2.0	1.7	0.0
Max	20.0	22.0	0.4

2.3.2 Determination of sample interval

While the correlation coefficients between the average numbers of hens from the one minute sampling interval and all simulated sample intervals were clearly above the set limit, concerning percentages of time spent with exploratory behaviour this was only the case for the sample intervals of two, three and five minutes. For dust-bathing behaviour only the sample interval of two minutes rendered results that correlated sufficiently with the one minute interval (Table 2.5).

Table 2.5: Spearmans` rank correlation coefficients for total numbers of hens and percentages of hens scratching & pecking and dust-bathing calculated on the basis of one minute sampling intervals in relation to two, three, five and ten minutes (n = 8)

r^{Spearman}	Sample interval 1 minute		
	Total numbers of hens	% of hens scratching & pecking	% of hens vertical wing shaking
Sample interval 2 minutes	0.976**	0.889**	1**
Sample interval 3 minutes	0.987**	0.701**	0.683**
Sample interval 5 minutes	0.987**	0.781**	No value calculable ¹
Sample interval 10 minutes	0.988**	0.501*	No value calculable ¹

¹No scratching occurred, *p = 0.05, **p = 0.01

2.3.3 Observation time

The correlation coefficients between the average numbers and percentages of hens calculated from the whole observation hour in relation to the simulated 16 and 30 minutes were above the set limit, except concerning percentages of hens performing exploratory behaviour (Table 2.6).

Table 2.6: Spearmans` rank correlation coefficients for total numbers of hens and percentages of hens scratching & pecking and vertical wing shaking on the basis of one hour observation time in relation to 16 and 30 minutes (n = 8)

r^{Spearman}	60 minutes		
	Total number of hens	% of hens scratching & pecking	% of hens vertical wing shaking
16 minutes	0.954**	0.594*	1**
30 minutes	0.959**	0.698**	1**

*p = 0.05, **p = 0.01

2.3.4 Intra- and inter-observer reliability

2.3.4.1 Intra-observer reliability

The correlation coefficients between the average numbers and percentages of hens calculated from two observation sessions were above the set limit, except concerning percentages of hens searching (Table 2.7).

Table 2.7: Intra-observer reliability (Spearman's rank correlation coefficients) concerning total number of hens and non-evaluable hens and percentages of hens scratching & pecking, dust-bathing, searching and preening during 16 minutes observations with a sampling interval of 2 minutes for two observation sessions (n = 17)

r_{Spearman}	Observation session 1					
	Total number of		% of hens			
	hens	non-evaluable hens	scratching & pecking	dust-bathing	searching	preening
Observation session 2	0.841**	0.828**	0.817**	0.704**	0.300	0.731**

**p = 0.05

2.3.4.2 Inter-observer reliability

The correlation coefficients between the average numbers and percentages of hens observed by two observers were only above the set limit concerning total numbers of hens and non-evaluable hens (Table 2.8).

Table 2.8: Inter-observer reliability (Spearman's rank correlation coefficients) concerning total number of hens and non-evaluable hens and percentages of hens scratching & pecking, dust-bathing, and preening during 16 minutes observations with a sampling interval of 2 minutes recorded by two observers (n = 17)

r_{Spearman}	Observer 1				
	Total number of hens	Non-evaluable hens	% of hens		
			scratching & pecking	dust-bathing	preening
Observer 2	0.875**	0.716**	0.072	0.455	0.601*

*p = 0.05, **p = 0.01

2.4 Discussion

2.4.1 Direct observations in commercial flocks

The direct observations on-farm turned out to be unfeasible in both different aviary systems: First, the very high density and number of hens within the indoor and outdoor litter areas of flock 1 rendered it difficult to identify the behaviour performed by all animals within the observers' field of view. Additionally, the total number of hens could only be estimated. This was not the case with flock 2; whereas on average about 215 and 231 hens had been counted in flock 1 on 22 to 24 sqm litter area parts, it were only 15 in flock 2 on 7 to 12 sqm. Another problem was that the observer could not see the areas under the aviaries, where presumably more dust-bathing was occurring than in the open litter area due to litter accumulating over time and a higher light intensity from the lights installed under the lowest platform of the aviary. This problem, however, also concerned the observations from video recordings. Further difficulty was the highly apparent influence of the observer on the hens' behaviour in both flocks. Hens of flock 1 pecked very obtrusively at the observer and her equipment. In contrast, hens of flock 2 behaved very fearfully, avoided the observer and fled under the aviary system. Lickteig (2006) mentioned the same problems from direct observation: laying hens of a commercial flock of 5,700 birds in an aviary first withdrew from the observer and later decreased the distance and started to direct their behaviours towards the person. The author then used only video observations to avoid this observer influence. Hence, in both flock 1 and 2 the percentages of dust-bathing hens were presumably lower than under undisturbed conditions. In general, it cannot be expected to find very high percentages of hens performing dust-bathing, except at times of the day where peaks of the behaviour might occur.

The extent of problem had not been expected as direct observations have been applied in many previous studies to assess dust-bathing as well as scratching and pecking behaviour using continuous and time sampling. However, in these cases mostly very restricted numbers of animals (circa six to 16 hens per pen) in small test pens (circa five to 15 sqm) had been observed (e.g. Vestergaard 1982, Blokhuis 1984, Petherick and Duncan 1989, Vestergaard et al. 1990, Bubier 1996, Nørgaard-Nielsen 1997). Only the situation found in flock 2 was comparable in terms of bird numbers and density with previous small scale studies. On the other hand, Odèn et al. (2002) also analysed dust-bathing behaviour on-farm in aviaries using time sampling. Herd size and stocking density were comparable to the present study, but the observer stood outside the system, observing one third of the litter area that was directly behind the entrance. Due to very low light intensity indoors and fine-meshed chicken wire, this was not feasible in the present study. Additionally, observations then could have been carried out only for the litter area parts behind the doors and not over the whole litter area.

Although no reliability problems or measures to overcome problems were mentioned by Odèn et al. (2002), the own experience suggests that in general the risk of a substantial observer influence on the birds' behaviour is very high in commercial flocks with limited possibilities to habituate the birds to the observer. Moreover, the extent of influence will likely vary between flocks with varying responsiveness towards humans, leading to confounding. In addition, it appears very challenging to reliably record behaviour of so many birds under high

stocking densities, as also mentioned by Knierim and Winckler (2009a). Martin and Bateson (2007) suggested that the observer could hide or give an adaptation time to the animals observed. The latter was already done in this study, but was of limited effect. Also Martin and Bateson (2007) state that regardless of an adaptation time it is difficult to verify whether observed animals are influenced in their behaviour, because changes may be very subtle. Moreover, it is almost impossible to compare behaviour in the presence and absence of an observer, as different days or times of the day will also affect the behaviour performed. Therefore, results of direct observations should be interpreted with care. It is also unknown how long-termed possible behavioural changes may last and, moreover, this may be different for different flocks.

Huber-Eicher and Wechsler (1998) observed feather pecking in 30 and 31 hens per test pen with stocking densities of 12 and 13 birds per sqm. Also they mentioned the difficulty to observe the exact number of feather pecks in all hens per group, which underlines the constraint of direct observations. Consequently, Huber-Eicher and Wechsler (1998) counted repeated pecks from the individual as one peck using the all occurrence sampling method. Vertical wing shaking is more space-consuming than feather pecking and therefore easier to observe. Nevertheless, it remains a challenge under practical commercial conditions. Lindberg and Nicol (1997) also mentioned difficulties due to poor visibility of hens caged in different systems, which, the authors concluded, possibly resulted in an underestimation of the dust-bathing bouts. This risk especially applied to flock 1 with high numbers and densities of hens. Anyway, to be able to perform direct observations in large flocks of laying hens, the observation area must strictly be limited depending on stocking density. However, if direct observations are chosen as a recording method, it would still be necessary to test reliability of data recording by e.g. inter-observer reliability testing.

Another aspect is the sampling interval when a time sampling method is used. Mostly in the literature no information is given why a certain interval has been chosen. At the same time applied intervals vary widely, e.g. for dustbathing from 2 (Johnson et al. 1998) over 5 (Blokhuys 1984) to 15 minutes (Odèn et al. 2002). Martin and Bateson (2007) mention common sample intervals of 15, 20 or 30 seconds in laboratory studies and the need for longer intervals with field studies, especially with long recording sessions. As dust-bathing takes on average 20 - 35 minutes (Fölsch 1981, Vestergaard 1982, Van Liere et al. 1990), a longer sample interval appears sufficient. In contrast, exploratory behaviour may need shorter intervals because the duration of one peck and a scratching bout is brief, so that a shorter interval should be chosen. In direct observations sampling intervals are also determined by the time needed to finalise one scan sample, and failed observations cannot be repeated. The one minute sample interval chosen here for the direct observation was feasible, but produced a high work load with the high number of hens in flock 1.

2.4.2 Video observations in commercial flocks

The video recording equipment used in different commercial flocks should fulfil certain requirements: It should be easy to handle, to be installed, be cleaned and disinfected and cope well with very different light and space conditions. Relatively small and simple cameras like the used SANTEC colour cameras, proved to be suitable in this regard, although video quality of the Axis 221 Network Cameras was much better.

Problems regarding high stocking density (flock 1) and poorly observable areas under the aviary (flock 2) were similar to the direct observations. Cameras fitted under the aviary block produced videos that could partly not be analysed due to light reflections or blocked view by single birds.

Also in the other camera areas video quality was sometimes poor because of high dust concentrations, mites on the lenses and low light intensity. Guesdon and Faure (2008) also reported from difficulties to observe parts of the dust-bathing behaviour due to poor video quality evoked by low light intensity. Generally, identification of behaviour was more difficult with videos in comparison to direct observation with mostly a clear sight of the hens. However, it is an important advantage to be able to repeat video sequences for identification of behaviour, for training and reliability testing, especially under the challenging conditions in commercial flocks. Of course, video technique is bound to risks of malfunctioning of camera equipment leading to less video material than planned which should be considered in the design of studies involving video observations. In the current study about 53 (flock 2) to 80 (flock 1) percent of the installed cameras could be analysed. The comparably low percentage in flock 2 was due to the cameras positioned under the aviary.

Another problem was a too high number of birds in view to reliably record their behaviour. This was solved by putting a frame on the video screen in order to limit the number of birds. However, such a frame should be placed under the camera within the litter area and filmed so that the size of the observed area can be determined. At the computer screen this frame can then be copied at a transparency to ensure that always the same area is analysed when the same defined video picture size is used. A frame of two right-angled yard sticks building one sqm can be recommended.

The recording results from flock 1 and 2 show that in flock 1 indoors more dust-bathing was performed than in the winter garden (6 and 1 %). In contrast, no dust-bathing was observed in flock 2. However, for scratching and pecking the percentages of hens were lower in flock 1 than in flock 2 (9 and 12 %). This highlights that in a hen house different litter-related behaviours will not be performed evenly at any place of the litter area, but will preferably be carried out at places providing certain stimulating conditions. It will not be possible to film all these places and by this to achieve a representative sample of the complete litter area. Therefore, currently it appears impossible to compare commercial farms in terms of exploratory or dust-bathing behaviour on the basis of video observations. However, video observations can be used to investigate effects of single conditions such as litter quality (i.e. humidity, material type, etc.) or height on the quantitative expression of exploratory and the quantitative and qualitative expression of dust-bathing behaviour under commercial conditions.

2.4.3 Determination of sample interval

Instantaneous scan sampling (Martin and Bateson 2007) was chosen as recording and sampling method, although for behaviours with short duration such as pecking or scratching, continuous behaviour recording might be more suitable. However, it does not allow observing a high number of animals at the same time (because of limitation of human abilities) and it costs lots of time to observe a whole light day of 16 hours. Moreover, pecking and scratching are rather frequent behaviours that often occur in bouts. Therefore, it can be expected that the loss of information by using a time sampling method is limited. One aim of the present pilot study was to determine the longest possible sampling interval which still leads to sufficiently reliable results in order to increase recording efficiency. Correlation coefficients were within a very similar range ($r = 0.976 - 0.988$) regarding the total number of hens, which underlines that hens used the litter areas rather continuously during the observation time. Correlations of scratching and pecking in contrast decreased with longer sample intervals. For both pecking and scratching and dust-bathing behaviour only the two minute time sampling showed a high correlation with the one minute interval. Different results for dust-bathing would possibly have been achieved if it had occurred at higher frequencies, either because of different times of the day (it preferably occurs at midday) or more favourable conditions for dust-bathing than in the investigated flocks. However, as it is intended to record the behaviour on a wide range of commercial farms and to take into account the whole light day, the conclusion was drawn that a sampling interval of two minutes is most appropriate in terms of feasibility and trueness of results.

2.4.4 Observation time

Using a sample interval of two minutes, it made not much difference regarding the recorded total number of hens and percentages of dust-bathing hens whether a whole hour, half an hour or a quarter of an hour was observed ($r = 0.954$ to 1). This result might be due to very low percentages of hens performing dust-bathing behaviour as mentioned before. However, for scratching and pecking the correlation between the complete hour and the other time slots was much lower and not satisfactory, reflecting a higher variability in scratching and pecking over time. Despite the relatively low correlations ($r = 0.698$ and 0.594), it was considered that with a given total observation time an even allocation over the light day would bear the advantage that effects of possible confounders such as feeding times or humans attending the hen house were lowered by decreasing the likelihood that they just happen during a limited, blocked observation time (e.g. always two whole hours before midday and in the afternoon). Furthermore, samples from whole light days are better comparable between different farms with different managements despite of different light or feeding schedules. For further analysis the quarter hour observation time was used.

2.4.5 Intra- and inter-observer reliability

2.4.5.1 Intra-observer reliability

Intra-observer reliability was acceptable for all parameters ($r = 0.704$ to 0.841) except for searching which posed a reliability problem ($r = 0.300$). However, correlations were at the margin of acceptability according to the set limit ($r = 0.7$) for percentages of hens dust-bathing ($r = 0.704$) and preening ($r = 0.731$). Martin and Bateson (2007) mention that correlations of just close to $r = 0.7$ may not be accepted by some authorities and should preferably be well above this limit. However, for difficult measures that are important, $r = 0.7$ might be sufficient. On the other hand they state that there is no absolute limit above which results are acceptable. Nevertheless, the $r = 0.7$ is commonly seen as a well suited reference level (Martin and Bateson 2007, Knierim and Winckler 2009b, Meagher 2009) because the calculated coefficient of determination is $r^2 = 0.49$, meaning that just about 50 % of the variance in one set of scores is accounted for statistically by the other set of scores (Martin and Bateson 2007). Consequently it must be said that on the one hand measuring dust-bathing and preening behaviour could be assessed reliably here by one observer according to common standards, but on the other hand it should be the goal in further studies on the current topic to reach correlations well above $r = 0.7$.

In the case of searching behaviour, reasons for the difficulty were the high numbers of hens within the observation frame and that it was often vague if a hen searched or just looked around. This behaviour was therefore excluded from the study ethogram, although it cannot be ruled out that more practice may lead to better results.

2.4.5.2 Inter-observer reliability

While inter-observer reliability was acceptable regarding total number and number of non-evaluable hens ($r = 0.875$ and 0.716), with respect to percentages of hens performing the different behaviours no sufficient inter-observer reliability was reached ($r = 0.072$ to 0.601). This illustrates that it is even more challenging to reach acceptable inter-observer than intra-observer reliability.

One reason might have been the rather moderate agreement concerning the number of hens non-evaluable, because to calculate the percentages of hens performing a certain behaviour these values are subtracted from the total number of hens observed. Further, especially the high number of hens and density of flock 1 as well as poor video quality, as mentioned before, made a reliable recording more challenging. In addition, the two different observers may vary in their interpretation of the behavioural definitions and their observation capability. For inter-observer reliability it is especially important to clarify behavioural definitions (Knierim and Winckler 2009a). Possibilities to improve inter-observer reliability lie mainly in an extended observation practice or in simplifying the observation task, e.g. by minimizing the observation area or number of animals to be observed (Martin and Bateson 2007, Knierim and Winckler 2009a).

2.5 Conclusion

Direct observations have the advantage of easier identification of behaviours, but turned out to be unfeasible due to observer influence and the lack of opportunity to repeat observations as well as to test the intra-observer reliability. Possible measures to overcome these problems are a limitation of the observation area depending on stocking density and observing the hens from outside the hen house or hidden from their view. However, often structural and light conditions in practice will not allow for this.

Video observations may pose difficulties in terms of partly poor video quality and technical failure. Both should be considered in the experimental planning. It will not be possible to observe the complete litter area representatively, and consequently different farms on the basis of video recordings. However, video observations can be used to investigate possible effects of different conditions such as litter quality or height on the expression of litter directed behaviours. In the case of high stocking densities, the observation area must be restricted to a certain area. The advantages of video recordings are that they allow training, intra-observer reliability testing and sequence repetition during observation sessions.

A two minute time sample interval appears to be appropriate in terms of time efficiency and trueness of recordings. Reducing observation time is critical for the trueness of results. It was decided on logical grounds that a given observation time should be evenly distributed over the whole light day.

The results regarding intra-observer reliability showed that acceptable levels can be reached under the investigated conditions, although room for improvement also became apparent. However, inter-observer reliability was not acceptable.

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3. Exploratory and dust-bathing behaviour in laying hens kept in aviary systems – identification of influencing factors

3.1 Introduction

According to the German welfare regulation (Tierschutz-Nutztierhaltungsverordnung -TierSchNutzV 2006) aviaries are one type of possible housing systems for laying hens. They allow the birds to use the third dimension, thereby making more efficient use of space than barn systems (Bessei 1997). In the aviary systems, among others, a minimum of 250 sqcm litter area per hen or one third of the accessible floor area must be provided during at least two thirds of the lighting hours. The litter material must be a suitable, friable substrate in sufficient amounts in order to enable the hens to fulfill their species-specific needs, in particular to perform pecking, scratching and dust-bathing behaviour (TierSchNutzV 2006). Litter constitutes the most important resource regarding exploratory and dust-bathing behaviour (Hughes 1976, Fölsch et al. 1986, Gerken 1989). For instance, Nicol et al. (2009) found in choice tests that hens significantly prefer pens with additional foraging material (shavings), perch and nest box versus a wired pen without enrichment. Additionally, Merrill and Nicol (2005) observed that pecking and scratching occurred significantly more often on wood shavings than on string, rubber or wire surface in caged hens.

How exactly litter is used is affected by genetic effects, ontogeny including learning, diurnal rhythm, social factors, and environmental conditions including management (Gerken 1989). While pecking behaviour is already present at hatch (Kruijt 1964, Williams and Strungis 1979), scratching occurs from the third day of life onwards in complete order (Kruijt 1964). Dust-bathing behaviour needs until the fifth week of a chickens' life for its' complete development (Kruijt 1964, Williams and Strungis 1979), but some elements already occur within the first week (Kruijt 1964). Highlighting the importance of early learning, Johnsen et al. (1998) observed in laying hen chicks either reared on wire or on straw, or on sand and straw for the first four weeks of life, that the hens reared on wire showed significantly less dust-bathing during weeks 5 to 6 and 40 to 41 of life, although all hens were similarly kept on straw and with sand filled dust-baths after the first four weeks rearing period.

With respect to preferred substrates, a number of choice experiments were performed. For instance, in broiler chickens latency to access sand was shorter, number of enters and vertical wing shakes during dust-bathing were higher as well as time spent on sand, compared to wood shavings, rice hulls and a paper animal bedding product. Also rate of pecking behaviour and time spent pecking was highest on sand (Shields et al. 2004). Laying hens also preferred sand over wood shavings for dust-bathing in test pens, after animals were housed alternately in pens providing one or the other material to make hens familiar with both (Van Liere et al. 1990). Looking at the function of dust-bathing to remove feather lipids out of the plumage, peat and sand were assessed to be most effective as well as favoured by the hens, if provided as an alternative to wood shavings (Van Liere 1992). Petherick and Duncan (1989) had slightly differing results concerning sand, with young domestic fowl performing significantly more scratching on peat and wood shavings as well as pecking on peat compared to sand and sawdust. Nevertheless, more and longer dust-bathing bouts appeared on peat, followed by sand and further declining over sawdust to shavings. Here it might have played a role that the

hens were housed in pens with wood shavings and only put onto the four different substrates for 30 minutes daily over eight days. So familiarity with the different substrates differed, but the high suitability of peat and sand to remove feather lipids still may have contributed to the longer dust-bathing bouts on them. However, the motivation to dust-bath in the wood-shavings may also have been lower because they were accessible constantly. Additionally, the higher pecking rate Shields et al. (2004) observed on sand compared to shavings or other material was due to pecking and scratching preceding dust-bathing behaviour which must be separated from exploratory behaviour.

Moesta et al. (2008) showed that fresh and used wood shavings differ in their characteristics and in the amount of dust-bathing they elicit, with used but still friable wood shavings being more stimulating, probably due to their smaller particle size. In general it can be concluded that friable substrates are preferred for pecking and scratching (Schrader 2008), such as sawdust granules because of their round form and small particle size (Weitzenbuerger 2005). On the other hand, Huber-Eicher and Wechsler (1998) found more exploratory behaviour in hens provided with plastic blocks in comparison to plastic pellets. Further, they observed a lower feather pecking rate, if litter was provided as straw with long stalks compared to grounded straw. Possibly the object pecking interfered concerning the plastic blocks, which may be stimulated to greater extent compared to small particles. The results allow no response in how far the ground pecking and scratching were affected. Secondly, long stalked straw facilitates the hens rather to perform a diversified behavioural repertoire as plucking and tearing. The opportunity to manipulate material with the bill in different ways may have been more satisfying to the hens, which probably could spend more time on exploratory behaviours. In general hens might prefer fine materials such as sand and peat for dust-bathing and coarser material such as long-stalked straw for their exploratory behavior. Litter provided in hen houses consequently should provide both.

Regarding litter quantity little is known about the most suitable depth of litter. Moesta et al. (2008) did not find differences in number of hens dust-bathing and latency to dust-bathe with litter depths of two or 20 centimeters, but more vertical wing shakes were performed on the litter height of two centimeters, which increased on used wood shaving compared to fresh ones. The authors concluded that possibly it is less easy to bring the substrate into the plumage. This provides a weak indication that greater litter height might meet requirements better concerning dust-bathing.

A further question concerns the space requirements for pecking and dust-bathing behaviour in laying hens, on which only little information is available (Bessei 2010). Dawkins and Hardie (1989) found that the area covered during scratching behaviour was between 540 and 1005 sqcm per hen. Under commercial conditions stocking densities from 18 hens per sqm floor area as well as group sizes up to 6,000 and even more regarding flock sizes, are common and the question arises, if both space concerning factors have influence on the hens' quantitative performance regarding their exploratory and dust-bathing behaviour. Additionally the light intensity, often at low stage in hen houses to prevent feather pecking and cannibalism, as a dust-bath eliciting factor (Fölsch 1981, Vestergaard et al. 1990) should be investigated under on-farm conditions to identify its' impact on litter directed behaviours as the exploratory and dust-bathing behaviour.

Even though a considerable amount of information from experimental studies on suitable litter for pecking, scratching and dust-bathing is available, the legal requirements in this regard are rather vague and the actual effects of litter management on pecking, scratching and dust-bathing under commercial conditions have not yet been investigated. Therefore, the aim of the present study was to analyse the on-farm situation of 22 German commercial aviaries in terms of possible effects of litter material, height and humidity on the quantitative expression of exploratory and dust-bathing behaviour. Further, possible influences of group size, stocking density and light intensity were taken into account. Based on the results it was the final goal to derive management recommendations for laying hen farmers.

3.2 Animals, materials and methods

3.2.1 Aviary systems and flocks

From end of 2010 to beginning of 2012 altogether 47 farms with aviaries were visited. From these 22 aviary systems, comprising 18 tier-systems and four portal-systems from seven German states and different companies and types, were examined for the current study (Tables 3.1 and 3.2; for examples see Figure 3.1, Figure 2.1 and Figure 2.2 of Chapter 2). Different layer strains were kept: Eight flocks consisted only of Lohmann Brown, nine of Lohmann Selected Leghorn and Lohmann Brown, one of Lohmann Selected Leghorn, Lohmann Brown and Lohmann Tradition and four of Dekalb Weiß. In three flocks strains were mixed within one pen. In all but one flock the hens were beak trimmed. Three aviaries had winter gardens which were, however, not taken into account in the current study due to the low number and missing light intensity measures. In the investigation period the laying hens were in the last third of their laying period and aged between 55 and 78 weeks.

Table 3.1: Regional distribution of aviaries included in the study

State	Number of aviaries
Niedersachsen	4
Nordrhein-Westfalen	2
Bayern	1
Baden-Württemberg	10
Sachsen-Anhalt	2
Hessen	2
Thüringen	1

Table 3.2: Distribution of types of aviaries included in the study

Company	Type	Number of aviaries
Meller	No type description available	2
Rihs Agro AG	Bolegg 1 Eco easy	1
Salmét	High-Rrise 3	2
	Floor system with one platform	1
Fienhage	Easy 80/100	2
Vencomatic	Red-L	1
	Bolegg Terrace	2
Volito	No type description available	2
	Vita	1
	Compact	2
Farmer Automatic	Kombinatic	1
Big Dutchman	NATURA 70	1
	NATURA-Nova Twin	2
	NATURA-Nova	2

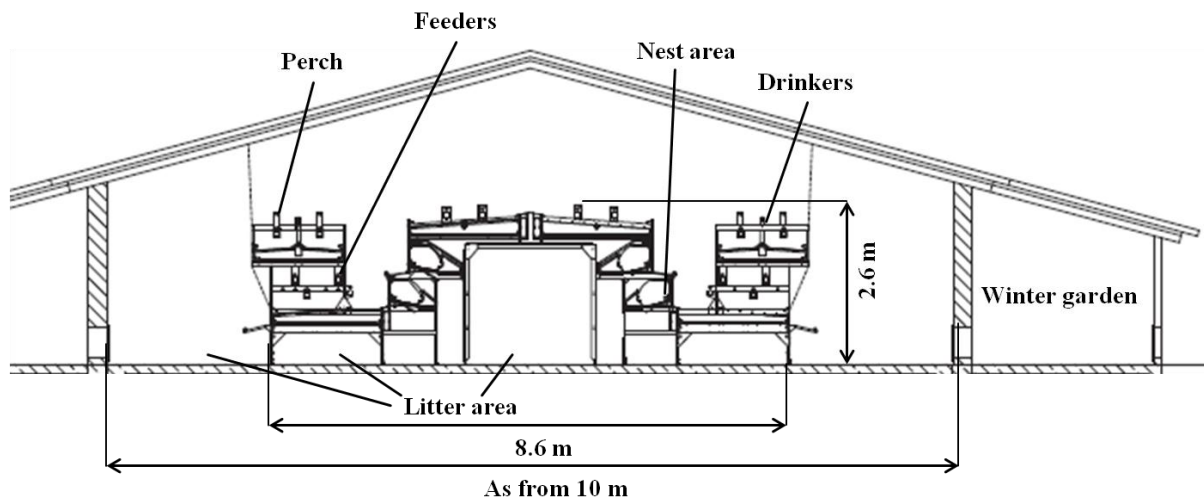


Figure 3.1: Example of portal-system: NATURA-Nova Twin aviary portal-system with winter garden to both sides (Big Dutchman 2012, own captions)

3.2.2 Video recordings and behavioural observations

Always four to six cameras (SANTEC colour cameras for corner mount (VTC-E220IRP) with IR-LEDs, 550 TVL, objectives with focal distance of 22° or 58°) were installed within the first pen of each aviary aiming to cover different conditions regarding litter height (high and low litter depth). Additionally ten to 12 cameras were installed filming different positions of perches, feeders and nest boxes so that 16 cameras at maximum were placed within the hen houses. Included into the current study were only video recordings from the cameras in the litter areas.

Cameras were fixed with wall arms and laces on walls or structural elements and connected to a common computer (for examples see Figures 2.3 to 2.5 of Chapter 2). Video recordings were saved on external hard drives (One Technologies, 1.5 TB). To allow analysis of always the same floor area size, a frame sized 1 sqm using two right angled yard sticks was filmed after camera installations. For optimal video quality and to ease video observations, if possible the cameras were installed at such a height that the video picture was equal to the 1 sqm area. Areas under the first platform of the tiers were filmed, too, if the recordings allowed observable videos. After camera installation, hens had one day to habituate to the technical equipment. The following two days were observation days. Poor video quality and camera failures led to a decreased number of video recordings. At maximum, recordings from five cameras could be analysed (6 farms), partly only from four (9 farms), three (2 farms) two (4 farms) or even only from one camera (1 farm). Instantaneous scan sampling (Martin and Bateson, 2007) every two minutes over the first 16 minutes of each lighting hour (eight sample points), distributed over two light days was carried out. If the light day started at an odd hour at the first observation day, all odd hours were analysed, and all even hours at the second observation day, and conversely. A light day ranged from 14 to 16 hours. Observations were carried out using The Observer® XT 10.5 Software (Noldus Information Technology, Wageningen, The Netherlands) for video playback and Excel for data entry (Microsoft Office Excel 2007). Always the first five seconds of every sample point were observed. In case behaviour regarding preening and dust-bathing was not clear, this was prolonged for further five seconds. If one sample point could not be analysed (e.g. due to a hen covering the lens) always the following sample point was analysed instead. Only those recordings were included in the analysis, in which at least 50 percent of the light hours were observable. Recordings from three cameras in two cases could only be analysed during eight light hours, in one case only nine instead of 15 light hours. Table 3.3 shows the assessed parameters and their definitions. In addition, preening and resting behaviour had been recorded, but was not included in the further analysis. Dust-bathing was only recorded if one of the listed movements was shown, because it was not possible to differentiate between resting-phase during a dust-bath and resting behaviour using the time sampling method. The pecking and scratching behaviour preceding a dust-bath was not counted to the exploratory behaviour and therefore not recorded. For video analysis the filmed yard stick frame was copied on a transparency, which was put over all videos of the corresponding camera at the computer. The area within the frame was analysed (Figure 3.2).

Table 3.3: Definitions of the assessed parameters in aviaries

Total number of hens	Sum of all hens that are completely or partly (with at least one claw, foot or other body part) located in the observation frame
Exploratory behaviour:	
Scratching	Number of hens performing fast movements with one foot or both feet alternately
Pecking	Number of hens performing a short, fast head movement towards the litter area floor
Dust-bathing behaviour:	Number of hens performing one of the following movements
Scratching and pecking/ squatting down/ bill raking	Hen performs many intensive scratches and pecks within a circumscribed area forming a dust-bath hollow after which the hen squats down and starts accumulating litter in front of her breast with her bill
Scratching with one leg	Hen lies on her ventral-lateral body side with fluffed feathers and scratches repeatedly and fast with her contra-lateral leg (modified after Van Liere and Wiepkema 1992)
Side rubbing	The hen lies on her side, one or both legs are stretched against the rim of the dust-bathing hole in the substrate and the hen rubs her body against the substrate (Moesta 2008, modified after Van Liere and Wiepkema 1992)
Wing and leg stretching	Hen lies on the side and stretches her upper completely opened wing backwards together with one or both leg(s) (Kruijt 1964)
Vertical wing shaking	The hen lies on her breast with fluffed feathers and shows fast vertical wing movements, immediately followed by rhythmic movements of the legs (Severin 2002, modified after Van Liere and Wiepkema 1992)
Axial body shaking	The hen stands and shakes her body around the long axis of the trunk with fluffed feathers and somewhat lifted wings in short accelerating movements (modified after Fölsch 1981, Moesta et al. 2008)
Total number of hens non-evaluable	Number of hens whose behaviour cannot be identified doubtlessly; for the calculation of the average percentages of hens performing certain behaviour at any time during the light day, these hens are subtracted from the total number of observed hens.



Figure 3.2: Screenshot of the observation frame (1 sqm) on a video picture opened with the Observer XT 10.5 (bodies of three hens outside the frame are blurry because hens were moving)

3.2.3 Further measures

Questionnaire guided standardized interviews with the farm managers were held to ascertain the layer strain and age, number of hens per flock at time of start of the laying period, if hens were beak trimmed, what kind of litter material was used (straw, wood shavings, saw dust, sand or other materials) and whether litter was provided repeatedly or not, as well as company and, if available, the type of the aviary system. The following measures were directly recorded: number of pens per aviary, pen size: size of the usable and the litter area according to the German welfare regulation (TierSchNutzV 2006), availability of litter material at the observation areas, litter height at the middle of each observation area, light intensity (measured with a Luxmeter in six directions corresponding to the six sides of a virtual cube and calculation of an average value; measured in front towards the door and in the middle to always the left and right side as well as under the system in front and in the middle and calculation of an average value for the six measure points). Also litter humidity and quality (if litter material was identifiable: yes or no) was scored. Humidity was assessed by trying to form a ball out of a handful of substrate and scoring of the result on a four-score scale (Table 3.4). From the data recorded the number of hens per pen and stocking density in the usable and litter area were calculated.

Table 3.4: Scoring of litter humidity after trying to form a ball from the litter

Dry	Substrate is dry and loose
Humid	Substrate sticks at the glove, but a ball cannot be formed
Cloddy	Substrate agglomerates
Wet	A persistent ball can be formed

3.2.4 Intra- and interobserver reliability and statistical analysis

Video data were analysed by two observers (Observer 1: 10 aviaries - 39 cameras, observer 2: 12 aviaries - 42 cameras). Intra-observer reliability for the measures total number of hens, percentages of hens non-evaluable, pecking, scratching and dust-bathing was tested by the chief investigator. After acceptable figures were reached, the second observer was trained and inter-observer reliability tested. Statistics used for reliability testing was the Pearson correlation coefficient as data were normally distributed (Kolmogorov-Smirnov-Test). Analyses were done in SPSS 19.0.0.1 (SPSS, IBM Company, Illinois). In line with Martin and Bateson (2007) values $r_{\text{Pearson}} \geq 0.7$ were regarded acceptable. Average values from eight sample points of one light hour from recordings from 15 cameras (intra-observer reliability testing) and 13 cameras (inter-observer reliability testing), respectively, originating from three farms constituted the independent samples.

Behavioural data were analysed descriptively by calculating means and standard deviations for total number of hens, percentages of hens observed, evaluable, pecking, scratching and dust-bathing (Microsoft Office Excel 2007). Mixed models using the “proc mixed” statement in SAS 9.2 (SAS Institute) were calculated for the total number of hens, percentages of hens evaluable, pecking, scratching and dust-bathing. Fixed factors were litter height (α_i), litter type (β_j), litter humidity (γ_k), pen stocking density (δ_l), group size (ϵ_m) and light intensity (ζ_n). Farms were set as random factor. Variance homogeneity and normal distribution of residues was given for all data. The models’ syntax was as follows:

$$y_{ijklmn} = \mu + \alpha_i + \beta_j + \gamma_k + \delta_l + \epsilon_m + \zeta_n + \eta_{ijklmn}$$

y_{ijklmn} = Dependent variable
 μ = Intercept
 η_{ijklmn} = Residuals

3.3 Results

3.3.1 Intra- and inter-observer reliability

Intra- and inter-observer reliability was assessed to be acceptable for all parameters (Table 3.5).

Table 3.5: Pearson correlation coefficients for intra-observer (n = 15) and inter-observer reliability (n = 13) concerning total number of hens and percentages of hens non-evaluable, pecking, scratching and dust-bathing

r_{Spearman}	Total number of hens	% of hens			
		non-evaluable	pecking	scratching	dust-bathing
Intra-observer reliability observer 1	0.996	0.964	0.952	0.833	0.771
Inter-observer reliability	0.932	0.706	0.958	0.871	0.964

3.3.2 Farm characteristics

Characteristics of the visited aviaries are displayed in Table 3.6. The different litter material used was categorized into three classes: missing, fine material, and fine material mixed with straw or pure straw (Table 3.7). One aviary was littered with two different fine materials (wood shavings and sand), the others had only one. In the aviaries where no litter material was provided ('missing'), still 'litter' heights of 0.5 to 17 cm (3.5 ± 4.1 cm, $n = 28$) were recorded due to accumulated dust and faeces. With fine material 2.6 ± 1.9 cm ($n = 26$) and with fine material mixed with straw or pure straw 3.0 ± 2.1 cm were assessed on average. In 2 cases, in contrast to the farmers' information, no litter material was found (litter height of 0 cm). Consequently, they were included in the category missing litter. According to the information provided by the farmers five aviaries were littered repeatedly. Of 12 observation areas (five aviaries) litter was found to be humid, 68 were dry. Only one area was found wet and allocated to the category 'humid' for the analysis, cloddy conditions were not found (Figure 3.3). In 52 observation areas of 14 aviaries litter was not identifiable (Figure 3.4).

Table 3.6: Characteristics of the 22 visited flocks in terms of flock and group size, number of pens per aviary, stocking density in the observed pen and litter area, size of litter area per pen, litter height and light intensity

	Min - Max	Mean \pm standard deviation	Median
Flock size (number of hens per aviary)	900 – 19,500	$6,883 \pm 5,892$	5,120
Group size (number of hens per pen)	400 – 6,000	$3,042 \pm 1,789$	3,015
Number of pens per aviary	1 – 12	2.5 ± 2.3	2
Stocking density (number of hens per sqm usable area)	6.1 – 18.5	10.4 ± 3.4	9.4
Stocking density in litter area (number of hens per sqm pen litter area)	10.9 - 76	27.7 ± 19.0	18.1
Litter area size (sqm)	22 – 1,002	303.6 ± 277.3	252
Litter height (cm)	0 - 17	3.1 ± 2.9	2.5
Light intensity (lx)	0.6 - 99.5	11.3 ± 16.3	6.5

Table 3.7: Distribution of observation areas (n=81) and aviaries in terms of categories of litter material used

	Missing litter (faeces and dust)	Fine material (sand, saw dust, wood shavings/chips, cellulose pellets)	Fine material (sand, saw dust, wood shavings/chips, cellulose pellets, chalk) and straw mixed or pure straw (in brackets)
Number of aviaries	8	7	7 (3)
Number of observation areas	28	26	27 (11)

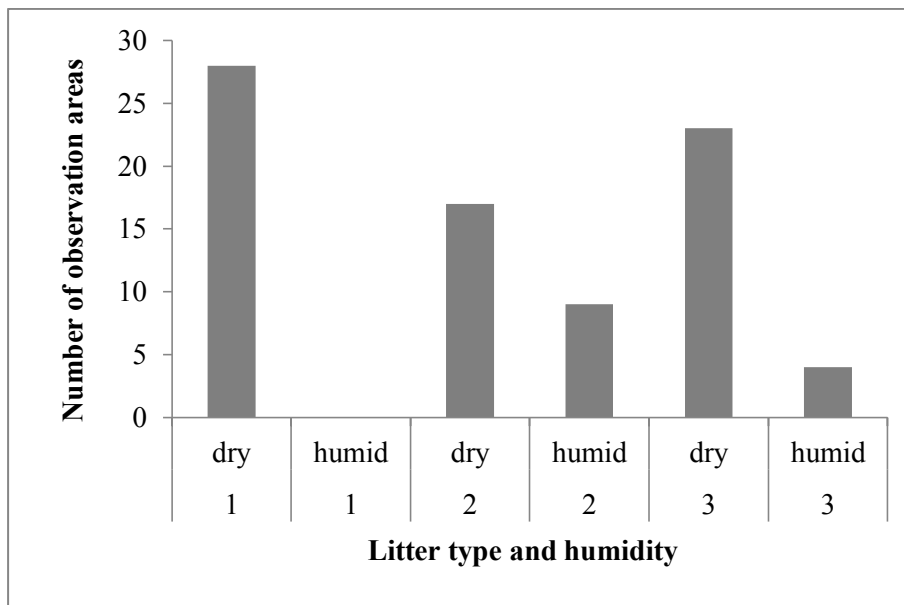


Figure 3.3: Distribution of observation areas in terms of litter humidity and the three litter categories (1 = missing litter, 2 = fine material, 3 = fine material mixed with straw or pure straw)

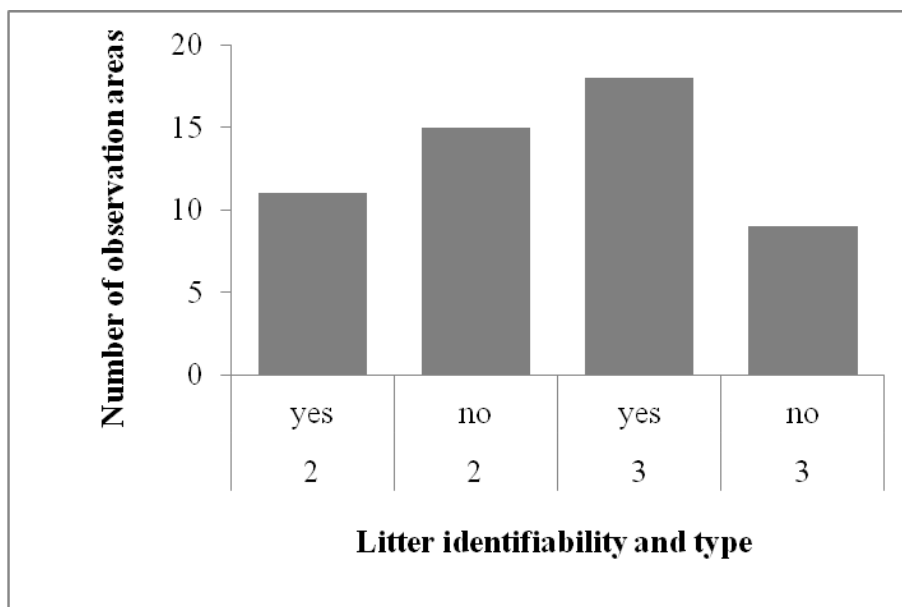


Figure 3.4: Distribution of observation areas in terms of litter quality (identifiable or not identifiable) and two litter categories (2 = fine material, 3 = fine material mixed with straw or pure straw)

3.3.3 Data overview and analysis of variance

On average 7.1 ± 4.5 hens were counted within the 1 sqm observation area (Figure 3.5). In 88.1 ± 9.8 % of the observed hens the actual behaviour performed could be identified. Most of the time hens performed pecking (24.7 ± 10.8 %). Other behaviours occurred less with on average 6.8 ± 7.3 % of hens dust-bathing and 2.2 ± 1.6 % scratching (Figure 3.6).

Analysis of variance (Table 3.7) revealed significant influences of litter height and humidity on scratching behaviour (Figure 3.7) as well as litter type on percentages of hens performing dust-bathing (Figure 3.8). Stocking density and group size were associated with the total number of hens within the observation area.

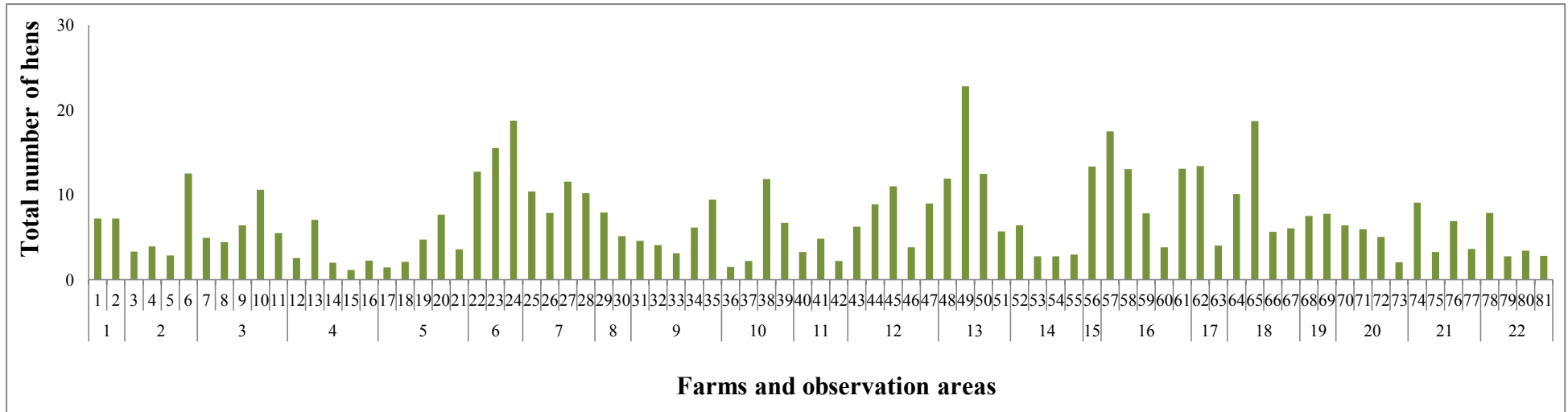


Figure 3.5: Total number of hens in the single observation areas (n = 81) distributed to the 22 farms

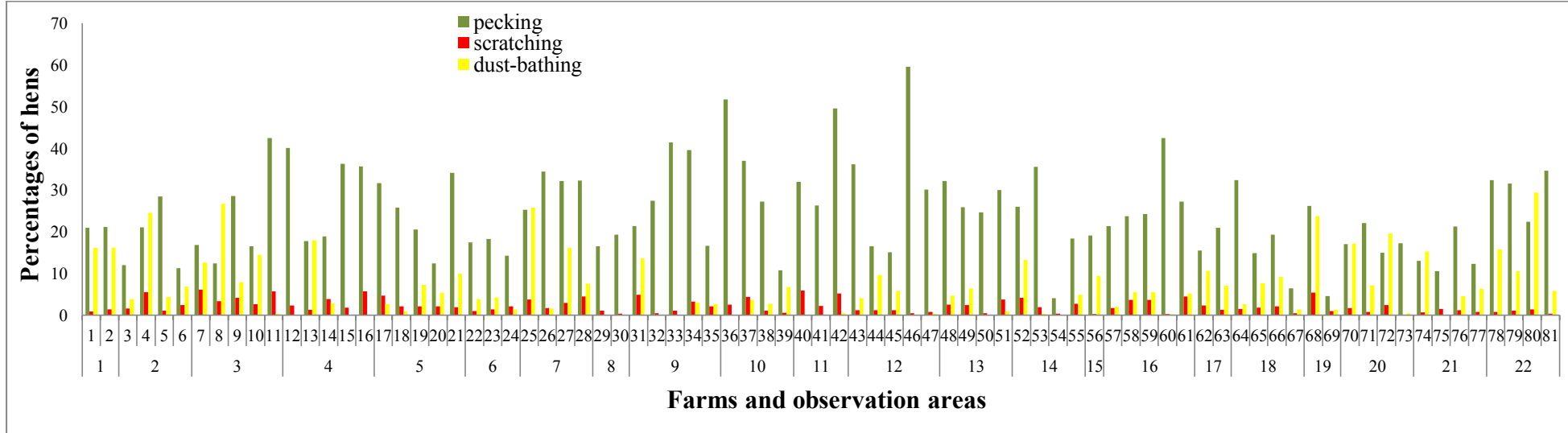


Figure 3.6: Percentages of hens pecking, scratching and dust-bathing in the single observation areas (n = 81) distributed to the 22 farms

Table 3.7: Results of analysis of variance concerning influencing factors on total number of hens, percentages of hens pecking, scratching and dust-bathing with farm set as random factor (n = 81, df = 72)

	Total number of hens			% pecking		
Fixed effects	Descriptor	p	F	Descriptor	p	F
Intercept	-0.01 ± 2.03	-	-	19.20 ± 5.21	-	-
Litter height (cm)	0.10 ± 0.17	0.5594	0.34	0.43 ± 0.43	0.3266	0.98
Litter type (1 - 3)	1 = -1.31 ± 1.28	0.1323	2.08	1 = 0.96 ± 3.28	0.2488	1.42
	2 = 1.25 ± 1.34			2 = 5.33 ± 3.43		
	3 = 0 (intercept)			3 = 0 (intercept)		
Litter humidity (dry, humid)	dry = 0 (intercept)	0.8625	0.03	dry = 0 (intercept)	0.7532	0.10
	humid = 0.24 ± 1.41			humid = 1.14 ± 3.61		
Stocking density (hens/sqm)	0.47 ± 0.14	0.0016	10.83	0.11 ± 0.37	0.7747	0.08
Group size (no. of hens/pen)	0.0007 ± 0.0003	0.0178	5.88	0.00 ± 0.00	0.5126	0.43
Light intensity (lx)	-0.03 ± 0.03	0.3796	0.78	-0.07 ± 0.09	0.6405	0.55
	% scratching			% dust-bathing		
Fixed effects	Descriptor	p	F	Descriptor	p	F
Intercept	2.41 ± 0.65	-	-	9.02 ± 3.37	-	-
Litter height (cm)	0.22 ± 0.06	0.0002	15.30	0.05 ± 0.28	0.8685	0.03
Litter type (1 – 3)	1 = 0.01 ± 0.41	0.8684	0.14	1 = -5.35 ± 2.13	0.0453	3.23
	2 = -0.19 ± 0.43			2 = -2.32 ± 2.22		
	3 = 0 (intercept)			3 = 0 (intercept)		
Litter humidity (dry, humid)	dry = 0 (intercept)	0.0088	7.26	dry = 0 (intercept)	0.4086	0.69
	humid = 1.22 ± 0.45			humid = -1.94 ± 2.33		
Stocking density (hens/sqm)	-0.05 ± 0.05	0.2733	1.22	0.17 ± 0.24	0.4858	0.49
Group size (no. of hens/pen)	-0.00 ± 0.00	0.8218	0.05	-0.00 ± 0.00	0.1798	1.84
Light intensity (lx)	0.01 ± 0.01	0.3303	0.96	0.01 ± 0.06	0.8555	0.03

1 = missing litter, 2 = fine material, 3 = fine material mixed with straw or pure straw, significance level: p = 0.05

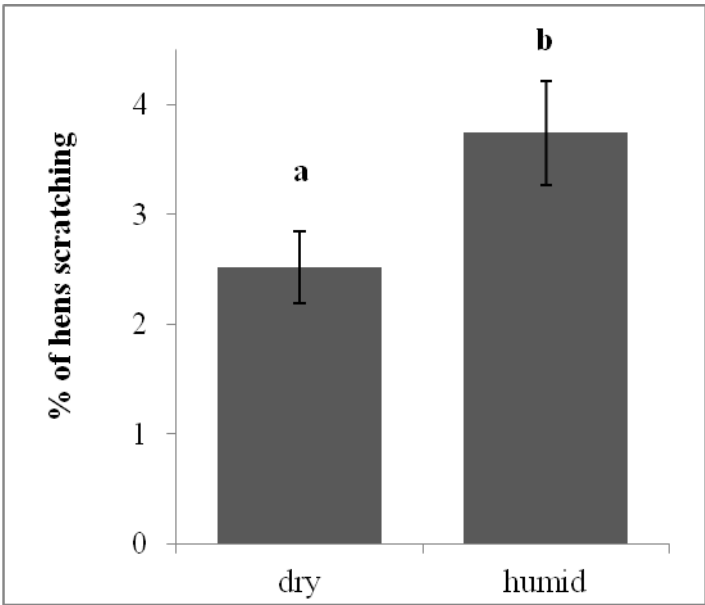


Figure 3.7: Percentages of hens scratching in dry or humid litter
 Different letters denote significant differences, $p = 0.05$ (least squares means of analysis of variance with farm set as random factor, $n = 81$, $df = 72$)

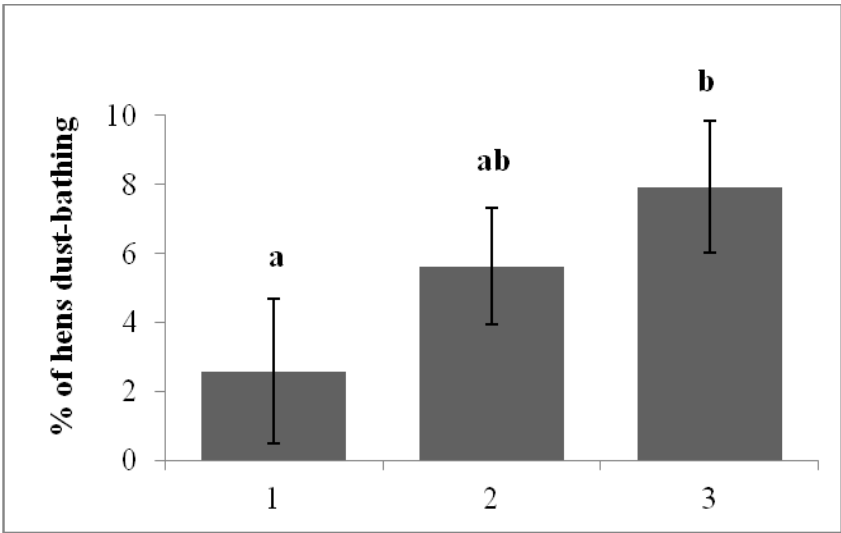


Figure 3.8: Percentages of hens dust-bathing in different litter types (1 = missing litter, 2 = fine material, 3 = fine material and straw mixed or pure straw)
 Different letters denote significant differences, $p = 0.05$ (least squares means of analysis of variance with farm set as random factor, $n = 81$, $df = 72$)

3.4 Discussion

3.4.1 Extent of exploratory and dust-bathing behaviour in the litter area

On average about 7 hens were found within the 1 sqm observation area. Taking into account that the hens were kept at a mean stocking density of 10 hens/sqm, it reflects an on average slightly uneven use of the accessible area, with less hens using the observed litter areas than the other areas. However, there was great variation between houses and camera positions (1 to 22 hens/sqm litter area over all observation areas). From these results, it cannot be said how many hens of the whole flock really visited the litter area, as the distribution of hens in the scratching area cannot be expected to be even. Broiler chickens, for example, were found to stay preferably near the walls, presumably to avoid disturbance by conspecifics (Buijs et al. 2010). It is well known that laying hens tend to gather at the edges of the tier systems during the night (Moesta 2007). Whether they also use the litter areas unevenly during the day has not been investigated systematically. If we, nevertheless, supposed an even distribution of hens in the litter area, the numbers found would mean that in the case of the analysed aviaries where one half of the accessible area was assessed to be littered, about 35 % of all hens were on average in the litter area. Lower proportion of 23 % was found by Carmichael et al. (1999) who counted hens in the litter areas of 10 pens of a large perchery system four times daily on three consecutive days, but the authors did not mention the proportion of the litter area and that the selection of observation areas was not intended to be representative for each farm. Instead, it was the aim within every farm to find observation areas that differed in the potentially influencing factors. Therefore, the figures obtained do not necessarily reflect how much time each hen spent in the litter area and with exploration and dust-bathing behaviour. In addition, further research is necessary to help to interpret the figures regarding use of the litter area in terms of animal welfare.

Hens pecked about 25 % of the observation time and scratched about 2 %. These results are very close to the ones from other studies in which 26 to 32 % of hens were pecking and 1.8 to 5.3 % scratching (Fitz 2007) or 25.6 % of hens pecking in the litter area (Baumgartner 2005). Also in further studies it has been found that hybrids spend only about 30 % of their time a day on feeding and foraging (reviewed by EFSA 2005). Feeding at the trough was excluded in the present study and the two other studies mentioned above. Research under near-natural conditions in a relatively small group of semi-wild Red Junglefowl showed that 60 % of the active part of a day was used for ground pecking and 34 % for ground scratching (Dawkins 1989). The substantially lower results found under commercial conditions may be caused by the less stimulating conditions of artificial compared to natural surroundings: Hens normally scratch intensively on the ground to recover particles to peck, which might not be necessary in hen houses with mostly one type of loose litter and a much smaller number of different stimuli in the litter. Materials used were usually of light structure, e.g. straw or sawdust. Apart from the general comparison between artificial and natural surroundings, it must be taken into consideration that in the present study there was a wide range in pecking activity between farms and observation areas (4 to 60 %). Thus, it is possible that not all farms provided adequate conditions to stimulate the hens sufficiently. For scratching, the variation between farms and observation areas was lower (0 to 6 %), partly due to the generally lower scratching

level. Here it appears that there is general room for improvement, although it cannot be ruled out that figures were biased due to the method of time sampling which might have caused an underestimation of the very short scratching instances. The fact that considerably higher percentages were found for dust-bathing than for scratching, which is against expectations, may underline this possibility. On the other hand, when looking through some of the video recordings continuously, it was striking that often hens were pecking for longer times without showing any intermediate scratching behaviour. This should be followed up in more detail in the future. At the same time, the significance of scratching for hen welfare is not well investigated. Huber-Eicher and Wechsler (1998) found in an experiment concerning the importance of scratching behaviour that for instance feather-pecking rates did not differ significantly between hens kept on either wood-shavings or wood-shavings covered with a wire. Thus, scratching opportunity does not appear to affect the occurrence of feather-pecking. On the other hand, the authors further found that pecking activity in the wire situation was significantly higher, which could have overruled possible effects of the scratching activity, because there is evidence for an association between the occurrence of feather-pecking and other pecking opportunity (Martin 1985, Huber-Eicher and Wechsler 1998, Olsson and Keeling 2005). Moreover, following the German welfare regulation (TierSchNutzV 2006) it is an obligation to enable the hens to perform all natural behaviours which includes not only pecking, but also scratching behaviour.

Dust-bathing behaviour was found to be performed on average during about 7 % of the observation time. This is in line with Fitz (2007) who found over the day 6.6 to 12.3 % of hens dust-bathing. This is clearly higher than the reference values calculated, based on the expectation that a hen normally dust-bathes every second day for about 20 - 35 minutes (Fölsch 1981, Vestergaard 1982, Van Liere et al. 1990) which corresponds to 1 to 2 % of a 16 hours light day. However, again the range between farms and observation areas was very high (0 to 29 %). Especially regarding dust-bathing it can be expected that it is not evenly performed in the whole litter area. It is well known that hens prefer to use certain areas for dust-bathing repeatedly and together with other hens (Reiter and Bessei 1999, Mench and Keeling 2001). Further, a tendency of litter to accumulate under the first platform was seen and it can be expected and was observed frequently that hens use these areas preferably for dust-bathing. Altogether, it is not possible to conclude from the samples observed on the extent to which dust-bathing was generally performed on the individual farms. To which extent the performance of exploratory and dust-bathing behaviour can be influenced by management factors will be discussed below.

3.4.2 Litter height

The average litter height of 3 cm found on the visited farms was below the few existing recommendations (Baumann 2006: 10 cm, diminished to 5 cm if external dust-baths of 20 to 25 cm depth are available; Bioland 2009: 5 cm). Eight houses (28 observation areas) were not littered at all, but covered with dust and faeces at an average height of 3.5 cm. Litter height partly varied largely within the same aviary, possibly due to uneven use by the hens. For example litter height ranged from 0 to 7 (fine material) in one aviary and in another from 6 to

17 cm (missing litter). This variation within and between farms allowed to analyse possible effects of litter height on exploratory and dust-bathing behaviour.

Scratching behaviour increased with increasing litter height in the present study whereas no significant influence on pecking and dust-bathing could be detected. Probably the higher litter attracted the hens more to recover the ground by strokes with their legs in search for consumable particles, which corresponds to the natural scratching behaviour (Kruijt 1964). Litter preferences by hens in connection to foraging behaviour have been rarely analysed in previous studies (LayWel 2006), especially not with regard to litter height. In general, no significant factor influencing pecking activity could be found, although percentages of pecking varied largely between the different observation areas. It must remain currently unexplained what the contributing factors were.

Regarding dust-bathing also Moesta et al. (2008) did not find any differences in duration on different substrate heights (2 versus 20 cm), only differences in the number of vertical wing shakes. However, as the dust-bathing sequences were not analysed in detail in this part of the study, it can only be concluded that apparently hens were able to adapt their dust-bathing behaviour to the different litter heights found under commercial conditions. This could have happened e.g. by a compensatory in- or decrease of the tossing phase. However, the closer analysis of selected dust-bathing events presented below (Chapter 5) did not reveal any influence of litter height on the proportion of the tossing phase or frequency of vertical wing shaking, but on the dust-bath duration which decreased with increasing height ($p = 0.0237$). On the first view this appears not to be in line with the present results. However, apparently increased dust-bath duration at higher litter heights was associated with a lower frequency of dust-bathing events, so that the proportion of time spent dust-bathing stayed about the same.

3.4.3 Litter type and quality

A great range of different litter materials and sometimes combination of litter materials was reported by the farmers. Combinations were mostly straw mixed with fine materials such as sand and wooden substrate (saw dust, shavings). However, mostly only one material was used. Where no litter material was provided, the areas were covered with dust and faeces, partly in high amounts.

Litter type had only a significant impact on hens dust-bathing, with 5.4 % more dust-bathing on fine material mixed with straw and pure straw compared to non-littered conditions. It is according to expectation that non-littered conditions are least attractive for dust-bathing. Moreover, it should be considered that observations were carried out in the last third of the laying period so that substrate could build up over time. In the beginning of the laying period, results might have been even more extreme because of completely lacking material. However, regarding the other litter types it was anticipated that especially fine material would elicit more dust-bathing than mixtures with coarser substrate, because preferences of hens for fine substrate are well established (Van Liere et al. 1990, Van Liere 1992, Bubier 1996, Merrill and Nicol 2005). However, also Fitz (2007) found hens to dust-bathe more in straw than in shavings. It must be kept in mind that the proportions of dust-bathing hens reflect both durations and frequencies of this behaviour. Thus, if hens dust-bathed longer, because it took them more time to work coarser material into the feathers, this would also affect the

proportions found. This will be confirmed in Chapter 5 where it will be presented that the average duration of the dust-baths was significantly lower on fine materials than on pure straw ($p < 0.0001$). Additionally, it cannot be ruled out that the litter type categories used here were too heterogeneous. However, the relatively small sample size did not allow greater differentiation. It would be worthwhile in the future to investigate this aspect of litter quality under commercial conditions in more detail with a more specific sample selection, including a separate category comprising litter areas with pure straw. Nevertheless, the current results indicate that the hens adapt their dust-bathing behaviour to the material provided, e.g. by elongating dust-bathing duration. Thus, it cannot be concluded from the quantitative results that any material was more suitable for the hens than the other. For this it would be necessary to analyse the dust-bathing sequences in more detail (see Chapter 5), and, even more importantly, to determine the functional effect of the different substrates on plumage condition.

In six littered aviaries (24 observation areas) no original material was identifiable, which may indicate that they were not littered regularly, while the original material was already at a high stage of degradation. However, in cases where fine material was used, it may well be that degradation occurs very rapidly. According to the farmers' information five aviaries were littered repeatedly during the laying period, but in three of these no litter was found in any observation area and litter of the remaining houses were not identifiable. This highlights the general need to use as far as possible data that have directly been recorded and to rely as little as possible on narrative information. Additionally, it appears that littering frequency is generally rather low. An increase may be one relatively easy possibility to better stimulate exploration behaviour.

Litter quality (original material identifiable or not) was not entered into the statistical models because of its correlation with litter type ($r_{\text{Pearson}} = -0.57$), and because AIC, AICC and BIC values turned slightly worse when it was included. However, when included, identifiable litter was associated with increased dust-bathing (5.2 % more, $p = 0.025$), providing a possible further explanation why litter including straw led to higher dust-bathing percentages. If more farms using this litter type provided fresh litter during the laying period or in higher frequencies and may have removed degraded and soiled litter, the provision of new litter material may also have had a stimulating effect on dust-bathing.

At the same time it is contrary to expectations that litter type did not affect exploratory behaviour and number of hens in the litter area. As most observation areas (52) had non-identifiable litter, attractiveness of all conditions may have been similarly low. However, since variation in exploratory behaviour between observation areas was large, especially for pecking behaviour, further factors, not included in the analysis, must have influenced the results.

3.4.4 Litter humidity

It is remarkable that in all cases of missing litter the areas were found to be dry. Thus, missing litter did not necessarily lead to increased humidity. Good ventilation systems and the hens' manipulation of dust and faeces with claws and bill were obviously sufficient to maintain dry and friable litter conditions. Looking at all observation areas only 13 were assessed to be humid, which is relatively low. Odén et al. (2002) and Moesta (2007) state that with high stocking densities litter may become humid and sticky, and a hard coat can build up on the floors. This was only the case in four observation areas here, which nevertheless were dry areas. Consequently, litter humidity constituted no major problem on the visited farms. This could be the reason why no effect was found on pecking and dust-bathing behaviour, contrary to expectations (Petherick and Duncan 1989, Van Liere et al. 1990, Sanotra et al. 1995, Shields et al. 2004, de Jong et al. 2005, Olsson and Keeling 2005, de Jong et al. 2007, Schrader 2008). Only scratching behaviour was significantly influenced by litter humidity with more scratching being found on humid areas. Possibly this occurred due to the fact the hens had to use their claws to remove the harder surface in some observation areas. This certainly does not mean that it should be the aim to keep litter humid, as negative effects of humid litter on bird health, especially with regard to infectious disease and foot problems are well-known. Instead it shows that higher behavioural frequencies do not necessarily mean that the conditions are more favorable for the hens in general, but that they adapt their behaviour to the specific conditions in the way it was already discussed above for dust-bathing and litter type.

3.4.5 Stocking density and group size

Maximum group sizes and stocking densities found on the commercial farms corresponded to the legal limits of 6,000 laying hens per group and 18 hens per sqm floor area (TierSchNutztV 2006). Average stocking densities with 10 hens per sqm were, however, lower than expected. Nevertheless, stocking densities with regard to littered area sometimes reached critical levels of up to 76 hens per sqm. Such densities arose because parts of the littered areas were not accessible to the hens in order to prevent them from laying eggs in the litter. Lickteig (2006) counted in a flock of a commercial aviary significantly more hens in the litter area when it was completely accessible for the hens (34 % of all hens) compared to a condition when the areas under the aviary were closed in order to prevent floor eggs (23 %).

Increased stocking density and group size both led to more hens in the observed litter areas. For stocking density this appears to be logical as the presence of more hens per area will also affect the litter area. However, Carmichael et al. (1999) found fewer hens in the litter area of a perchery system with increasing stocking density, ranging from 9.9 to 19.0 hens/sqm (four test pens), thus regarding comparable stocking densities to the current study. The authors state, that a lack of resources may decrease the number of animals demanding for them. On the other hand, they only found a decreased use of the litter area, whereas resources such as perches were not influenced. Differences between the studies may be due to differences in the design of the aviaries. However, also in the current study the increase in stocking density did

not lead to a proportional increase in the litter area, as every additional hen/sqm available area did only lead to an increase of 0.5 hens/sqm litter area.

The effect of group size cannot be unanimously explained. One possibility is that other areas in the house were avoided to a greater degree with larger groups, e.g. the areas on the tiers around feeders and drinkers in order to avoid conspecifics and agonistic interactions, or that the hens were more strongly attracted to the litter areas due to social facilitation. For instance, Reiter and Bessei (1999) found significantly more boiler chickens scratching and a higher feeding activity with increasing group sizes. On the other hand, here no significant influences on exploratory behaviour could be detected. Indeed Carmichael et al. (1999) found fewer hens dust-bathing with increasing stocking density, whereas no effect was apparent in the current study. The hens being present in the litter areas must have spent their time on other behaviours than exploration or dust-bathing, such as moving to other aviary parts, preening, being idle or else.

3.4.6 Light intensity

Light intensity was on average 11 lx. According to the literature maximum illuminances of 10 lx shall serve to lower the activity of both layers and broiler chickens (Appleby et al. 1992, EFSA 2005) and help to prevent feather pecking and cannibalism (Kjaer and Vestergaard 1999, EFSA 2005).

Light intensity did not have any significant impact on the assessed parameters. This is against expectations as especially dust-bathing can be elicited by light (Fölsch 1981, Vestergaard et al. 1990). Prescott and Wathes (2002) observed laying hens to eat preferably at 200 lx if feed was given mixed with sand or gravel compared to < 1 lx. Manser (1996) recommends light intensities of 20 lx at minimum to prevent inactivation of behaviour. This goes along with Davis et al. (1999), who found hens to prefer highest light intensity for active behaviours and lowest (6 lx) for resting. Possibly the light was at too low stage to influence hens' behaviour here. On the other side, a great range in light intensity was found between the farms (1 – 100 lx), which would lead to the assumption that there was enough variance of that factor to detect any influence. However, in most farms (14) 0 to 10 lx were measured in the litter area and only in two more than 30 lx, the other six lay in between. In further studies on this topic more attention should be paid to a sufficient variation in light intensity by choosing more heterogeneous farms regarding this factor. Further, for the current analysis average values from the litter areas in general were used. It would have been more appropriate to use the exact light levels of the single observation areas.

3.5 Conclusion

Within the range of conditions found on the investigated commercial laying hen farms with aviary systems no factor could be identified that affected the amount of pecking behaviour. Concurrently, the proportions of time hens performed pecking in the different litter areas observed over the light day varied largely between 4 and 60 %. Thus, obviously certain characteristics of the litter areas that have not or not appropriately been taken into account

favour the performance of pecking behaviour at activity levels that are closer to the figures found under semi-natural conditions than the average values of about 25 % recorded here.

Scratching levels were generally found to be low which partly may have been due to the time sampling method applied. However, increased levels were recorded in litter areas with higher litter. Indeed, litter height was mostly found to be low with 2.5 cm on average. While it was also found that hens scratched more in humid litter, probably reflecting a simple behavioural adaptation to the environmental conditions, of course still litter should be kept dry for general health reasons.

The extent of dust-bathing behaviour was observed to be influenced by litter type with least dust-bathing in areas where no litter material had been provided and only dust and faeces constituted the substrate to be used. While litter material including straw was associated with most dust-bathing behaviour, the meaning of the finding is not clear yet. It could reflect a higher attractiveness of this kind of material, but also a need to spend more time working the coarser material into the plumage.

The non-representative selection of observation sites per farm does not allow any firm conclusion on the exploration and dust-bathing levels on the individual farms, but it generally appears that the amount of pecking and scratching behaviour on average was rather low. Thus, measures should be taken to provide a more exploration stimulating environment, possibly such as providing new litter material more frequently. The results presented above underline the legal requirement in force that some kind of litter material must be provided which obviously is not current practice on all farms. Moreover, amounts of litter on the floor should be increased. Which exact litter height and litter type can be recommended needs further research, as well as how to better stimulate pecking behaviour.

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4. Exploratory and dust-bathing behaviour in laying hens kept in furnished cages – identification of influencing factors

4.1 Introduction

Since 2009 battery cages are banned by the German welfare regulation (TierSchNutzV 2006) and improved furnished cages (so-called small group housing systems) are the only allowed cage systems in Germany. They must provide at least 90 sqcm scratching area per hen to enable them to exercise their species-specific maintenance behaviours, especially pecking, scratching and dust-bathing, as undisturbed as possible (TierSchNutzV 2006, Bessei 2010). To this end, the systems are equipped with synthetic mats that shall be littered regularly with feed, bran, sawdust, wood shavings or pellets, chopped straw, straw pellets, peat or sand (EFSA 2005, Schrader 2008). Mats have an artificial turf or flat surface, which can be perforated. For hybrid hens kept in cages, Hughes (1976) found in choice tests a strong preference for litter, which was more distinctive with hens housed on wire than in hens housed in littered pens. Further, Merrill and Nicol (2005) observed that pecking and scratching occurred significantly more often on wood shavings than on wire wrapped with garden twine, perforated rubber matting or pure wire surface in caged hens. Both results indicate that caged hens prefer loose, friable litter for the execution of pecking and scratching behaviour. However, regarding the importance of litter for dust-bathing a number of different studies came to controversial conclusions: Colson et al. (2007) reared laying hens first in one unfurnished floor pen, one furnished floor pen and in one rearing aviary, all of them littered. At start of lay hens of the unfurnished floor system were transferred to non-littered cages and the others to littered laying aviaries. After hens were housed 36 to 43 weeks in the new systems they were transferred into sawdust-littered test arenas. The caged hens showed shorter latencies to dust-bathe, longer and more frequent dust-baths and more hens dust-bathed compared to hens housed in aviaries, indicating a higher dust-bathing motivation in the deprived hens. In contrast, Guesdon and Faure (2008) did not find differences in the dust-bathing of laying hens that were kept in different cage systems with either access to substrate or not after being reared in floor pens. After at least 15 weeks kept in the laying cages hens were tested for their latency to dust-bathe and the number of hens performing vertical wing shakes during 50 minutes in test cages littered with sawdust on five consecutive days. Furthermore, hens, which had no access to substrate in their home cages showed no displacement activities. Their latency to dust-bathe and the number of hens dust-bathing did not differ significantly between the groups, the latencies decreased similarly for all test groups over the test days. In addition, Faure (1991) did not find that hens were ‘working’ for access to litter if reared in battery cages or floor pens. He concluded that hens do not have a demand for litter material. On the other hand, Gunnarsson et al. (2000) found hens reared in floor pens and caged after one year of age working for access to straw. However, hens used the substrate for scratching and pecking, but not for dust-bathing in the test situation. These results go along with Wichman and Keeling (2008), who found hens working for access to peat, independently if they had previous experiences of litter or not. Widowski and Duncan (2000) found a tendency in hens to work more for the access to litter (peat moss) when they were litter deprived in comparison to hens that were non-deprived. Furthermore the deprived hens

performed significantly more dust-baths, suggesting that they were very motivated to dust-bathe. Petherick et al. (1990) applied also a choice test, where hens could obtain weather access to a wired floor or peat. Hens always did chose the peat variant, but the scientists did not find a general preference of the hens to perform preferably dust-bathing or foraging behaviour on the substrate, suggesting that hens had individual motivation regarding litter use. Lindberg and Nicol (1997) observed significant longer dust-bath durations and elements directed to the substrate (sand) as pecking and rubbing more frequently in the dust-baths of two modified cage systems than on the wired floor of conventional and modified cages, supposing that dust-bathing was more appropriate when performed in the dust-baths. Hence, to which extent caged laying hens have a demand for litter for dust-bathing behaviour is still scientifically debated. Nevertheless, the German welfare regulation (TierSchNutzV 2006) requires an adequate scratching area for both exploratory and dust-bathing behaviour.

In contrast to the great number of experimental studies regarding the significance and use of litter areas in cage systems, up to date there is less information available concerning the current on-farm situation. The first goal of the present study therefore was to give an overview on the status quo regarding scratching areas based on 16 commercial German laying hen farms with furnished cages. This concerns mat equipment (litter availability and material, mat number and mat dimensions) and the percentages of hens on mats and time spent with exploratory (pecking and scratching) as well as dust-bathing behaviour.

Furthermore, it was the aim to identify influencing factors on the quantitative expression of exploratory and dust-bathing behaviour on the scratching mats, taking into account availability of litter on the scratching mats, number of scratching mats per cage, mat width, and mat area per hen, cage stocking density, group size and light intensity.

4.2 Animals, materials and methods

4.2.1 Housing systems and flocks

From end of 2010 to beginning of 2012 17 farms with furnished cages according to the German welfare regulation (TierSchNutzV 2006) from all over Germany were visited. One farm was excluded because of missing scratching mats so that data from 16 farms from eight states were included in the present study (Table 4.1). Cage systems were of different types and from different companies (Table 4.2, picture examples in Figures 4.1 and 4.2). Seven flocks comprised only Lohmann Brown hens, one Lohmann Tradition and one Lohmann Selected Leghorn, and seven Lohmann Brown as well as Lohmann Selected Leghorn hens. Lines were not mixed within one cage. Eight flocks were beak trimmed. During data collection hens were in the last third of their laying period, between 53 and 78 weeks of age.

Table 4.1: Regional distribution of furnished cages included in the study

State	Number of furnished cages
Schleswig-Holstein	1
Niedersachsen	4
Nordrhein-Westfalen	4
Bayern	1
Baden-Württemberg	2
Sachsen	1
Sachsen-Anhalt	2
Hessen	1

Table 4.2: Distribution of types of furnished cages included in the study

Company	Type of cage	Number of farms
Big Dutchman	EV-1500-EU-60	2
	EV-1500a-EU-60	4
Farmer Automatic	Maxi System	2
Hellmann	No type description available	3
Salmet	4000/735	3
Specht	Varia	1
Zucami	Kleinvoliere	1

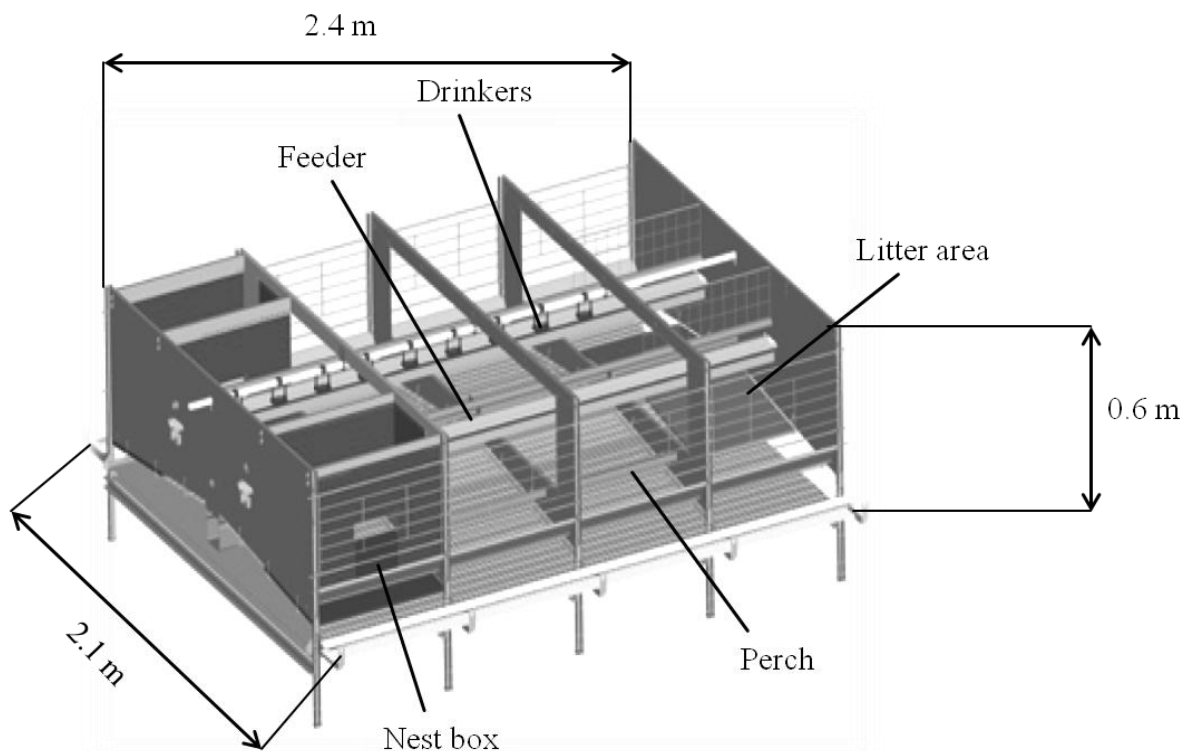


Figure 4.1: Farmer Automatic Maxi System (Farmer Automatic 2012, own captions)



Figure 4.2: Furnished cage system from Zucami with 8 floors (own picture)

4.2.2 Video recordings and behavioural observations

In every laying hen house four cages were equipped with 4 cameras each (SANTEC colour cameras for corner mount (VTC-E220IRP) with IR-LEDs, 550 TVL, objectives with a focal distance of 22° or 58°). One camera per cage was directed on one scratching mat, and additionally one camera at the feeding trough, perch and nest area. The current study only takes into account the cameras towards the scratching mats. Cameras were fixed with wall arms and laces on structural elements and connected to a common computer (Figure 4.3). Videos were saved on external hard drives (One Technologies, 1.5 TB). Cameras were always installed in the second tier in the right cage part of the third and sixth cage and in the left cage part of the fourth and seventh cage. In case of housings with cages installed in more than one row, always the cages of one row towards a building wall were filmed, which was most easy to equip with the camera equipment. For 11 farms video recordings from all four cameras could be analysed, for three only three and for two only two because of poor video quality and camera failure, respectively. After camera installation, hens had one day to habituate to the technical equipment. The following two days were observation days. Instantaneous scan sampling (Martin and Bateson, 2007) every two minutes over the first 16 minutes of each lighting hour (eight sample points), distributed over two light days was carried out using The Observer® XT 10.5 Software (Noldus Information Technology, Wageningen, The Netherlands) and Excel for data entry (Microsoft Office Excel 2007). If the light day started at an odd hour at the first observation day all odd hours were analysed and all even hours at the second observation day and conversely. Light days ranged from 13 to 16 hours. Always the first five seconds of every sample point were observed. If the behaviour was not clearly recognizable, observations were prolonged for five further seconds but not concerning pecking and scratching, which had to be recognizable within the first five seconds. If one sample point could not be analysed (e.g. due to a hen covering the lens) always a following sample point was analysed instead. For one camera only eight hours instead of 14 complete

light hours could be observed. Table 4.3 shows the assessed parameters and their definitions. Dust-bathing was only recorded when one of the listed movements was shown because it was not possible to differentiate between a resting-phase within a dust-bathing bout and other resting behaviour using the time sampling method. Preening and resting were additionally assessed, but not included in the further analysis.



Figure 4.3: Position of camera directed at scratching mat in the furnished cage system KV 1500 from Big Dutchman (own picture)

Table 4.3: Definitions of the assessed parameters in furnished cages

Total number of hens	Sum of all hens that are completely or partly (with at least one claw, foot or other body part) located on the scratching mat
Exploratory behaviour:	
Scratching	Number of hens performing fast movements with one foot or both feet alternately on the scratching mat
Pecking	Number of hens performing a short, fast head movement towards the scratching mat
Dust-bathing behaviour:	Number of hens performing one of the following movements
Scratching and pecking/ squatting down/ bill raking	Hen performs many intensive scratches and pecks within a circumscribed area on the mats after which the hen squats down
Scratching with one leg	Hen lies on her ventral-lateral body side with fluffed feathers and scratches repeatedly and fast with her contra-lateral leg (modified after Van Liere and Wiepkema 1992)
Side rubbing	The hen lies on her side, one or both legs are stretched and the hen rubs her body (modified after Moesta et al. 2008 and Van Liere and Wiepkema 1992)
Wing and leg stretching	Hen lies on the side and stretches her upper completely opened wing backwards together with one or both leg(s) (Kruijt 1964)
Vertical wing shaking	The hen lies on her breast with fluffed feathers and shows fast vertical wing movements, immediately followed by rhythmic movements of the legs (Severin 2002, modified after Van Liere and Wiepkema 1992)
Axial body shaking	The hen stands and shakes her body around the long axis of the trunk with fluffed feathers and somewhat lifted wings in short accelerating movements (modified after Fölsch 1981, Moesta et al. 2008)
Total number of hens non-evaluable	Number of hens whose behaviour cannot be identified doubtlessly; for the calculation of the average percentages of hens performing certain behaviour at any time during the light day, these hens are subtracted from the total number of observed hens.

4.2.3 Further measures

Questionnaire guided standardized interviews with the farm managers were held to ascertain the layer strain and age, number of hens per flock and hens per cage at time of start of the laying period, if hens were beak trimmed, what kind of litter material was used (feed, wood shavings, sand or other materials) as well as company and if available type of furnished cage system. The following measures were directly recorded: number of cages per flock, cage size (usable area according to the German welfare regulation TierSchNutzV 2006), scratching mat number per cage, length, width and type of mat (synthetic turf or other type, examples see Figure 4.4). Further, light intensity at the filmed mat was measured with a luxmeter in six directions corresponding to the six sides of a virtual cube and an average value calculated. Additionally assessed, but not included in this study were mat colour (green, brown or other colour). Mats were regarded as littered if litter could be detected (yes: litter is available or no: no litter is available). From the data recorded the stocking density in the cages (number of hens per sqm usable cage area), total scratching mat area, area of one mat and mat area per hen were calculated.



Figure 4.4: Upper picture: Mat with unperforated flat surface; middle picture: perforated flat surface; lowest picture: synthetic turf (own pictures)

4.2.4 Intra- and inter-observer reliability and statistical analysis

Video data were analysed by two observers (observer 1: 10 hen houses – 34 cameras, observer 2: 6 hen houses – 23 cameras). Intra-observer reliability for the measures total number of hens, percentages of hens non-evaluable, pecking, scratching and dust-bathing was tested by the chief investigator. After acceptable figures were reached, the second observer was trained and again intra-observer as well as inter-observer reliability tested. Average values from eight sample points of one light hour from recordings from 14 cameras, originating from four farms constituted the independent samples. Agreement of results between or within observers was tested using Spearman's rank correlation analysis in SPSS 19.0.0.1 (SPSS, IBM Company, Illinois). The limit for acceptable reliability was set at $r_{\text{Spearman}} \geq 0.7$ (Martin and Bateson 2007).

Behavioural data were analysed descriptively by calculating means and standard deviations for the percentages of hens on the total mat area and hens evaluable, pecking, scratching and dust-bathing (Microsoft Office Excel 2007). For calculating the percentages of hens on the total mat area, the number of observed hens on the film mats was multiplied with the number of mats per cage and divided by the number of hens per group multiplied with 100.

Mixed models using the "proc mixed" statement in SAS 9.2 (SAS Institute) were calculated for the total number of hens, percentages of hens pecking, scratching and dust-bathing. Fixed effects were litter availability (α_i), number of mats per cage (β_j), mat width (γ_k), mat area per hen (δ_l), cage stocking density (ϵ_m), group size (number of hens per cage, ζ_n) and light intensity (η_o). Farms were set as random factor. Variance homogeneity and normal distribution of residues was given for all data. The models' syntax was as follows:

$$y_{ijklmno} = \mu + \alpha_i + \beta_j + \gamma_k + \delta_l + \epsilon_m + \zeta_n + \eta_o + \theta_{ijklmno}$$

$y_{ijklmno}$ = Dependent variable

μ = Intercept

$\eta_{ijklmno}$ = Residuals

4.3 Results

4.3.1 Intra- and inter-observer reliability

Intra- and inter-observer reliability was acceptable for all recorded parameters, but could not be tested for scratching behaviour because of no occurrences (Table 4.4).

Table 4.4: Intra- and inter-observer reliability (Spearman's rank correlation coefficients) for total number of hens and percentages of hens non-evaluable, pecking, scratching and dust-bathing (n = 14 cameras)

r _{Spearman}	Total number of hens	% of hens			
		non-evaluable	pecking	scratching	dust-bathing
Intra-observer reliability observer 1	0.999**	0.809**	0.780**	not observable	0.906**
Intra-observer reliability observer 2	0.907**	0.768**	0.949**	not observable	0.911**
Inter-observer reliability	0.959**	0.729**	0.755**	not observable	0.767**

**p = 0.01

4.3.2 Farm characteristics

Characteristics of the visited hen houses, in particular of the scratching areas in the furnished cages, are displayed in Table 4.5. All scratching mats within one furnished cage system had the same size. 13 farms used synthetic turf mats, two farms mats with perforated, but flat and one farm mats with unperforated flat surface (Figure 4.4). Litter was found on mats of nine cage systems, with litter material always being feed. Therefore, as feed is no litter, in the following the term 'substrate' will be used instead.

Table 4.5: Characteristics of the 16 visited flocks in terms of flock, group and cage size, cage number per flock, stocking density and scratching mat number, length, width, total area, area of one mat and area per hen, as well as light intensity in the scratching area

	Min - Max	Mean ± standard deviation	Median
Flock size (number of hens/farm)	300 – 93,797	20,248 ± 30,762	5,670
Cage number per flock	27 – 2,432	399 ± 714	79
Cage size (sqm)	1.9 – 5.4	4.05 ± 0.97	3.78
Group size (number of hens/cage)	26 - 60	47.4 ± 10.8	50
Cage stocking density (number of hens per sqm usable cage area)	8.6 - 14	11.6 ± 1.4	11.1
Number of mats per cage	1 - 4	-	2
Mat length (cm)	31 - 118	63 ± 24.2	60
Mat width (cm)	22 - 72	33 ± 11.5	33
Total mat area per cage (sqcm)	713 - 6,650	3,913 ± 1,551	3,700
Mat area of one mat (sqcm)	713 - 5,832	2,151 ± 1,206	2,160
Mat area per hen (sqcm)	22.3 - 110.8	81.9 ± 24.8	88
Light intensity (lx)	0 – 8.3	1.8 ± 1.9	1.3

4.3.3 Data overview and analyses of variance

On average 2.9 ± 1.5 of the hens of one group (cage) were present on the observed mat area. Supposing an equal distribution of hens on all mats this corresponds to 11.7 ± 4.8 (3.2 to 19.9) % of the hens of one cage on the total mat area. In 92.1 ± 5.0 % of the observed hens the behaviour performed could be identified. The average hen spent 8.1 ± 5.4 % of the observed time pecking, 4.4 ± 4.1 % of time dust-bathing and 0.4 ± 0.5 % scratching (Figure 4.5).

Substrate availability was significantly associated with the percentages of hens on the total mat area and percentages of time the hens spent dust-bathing (Table 4.6). If substrate was available, 6.5 ± 1.9 % more hens were observed on the mats, with 8.1 ± 2.4 % more hens performing dust-bathing behaviour. With an increasing number of mats per cage the time spent pecking was reduced significantly. Mat width had a significant impact on dust-bathing behaviour, which increased with increasing width. An increasing mat and cage stocking density both significantly and positively influenced the percentages of time the hens spent pecking and scratching. Cage stocking density was also positively associated with the percentages of hens on the mats. Additionally, significantly higher percentages of hens were on the total mat area when light intensity was higher.

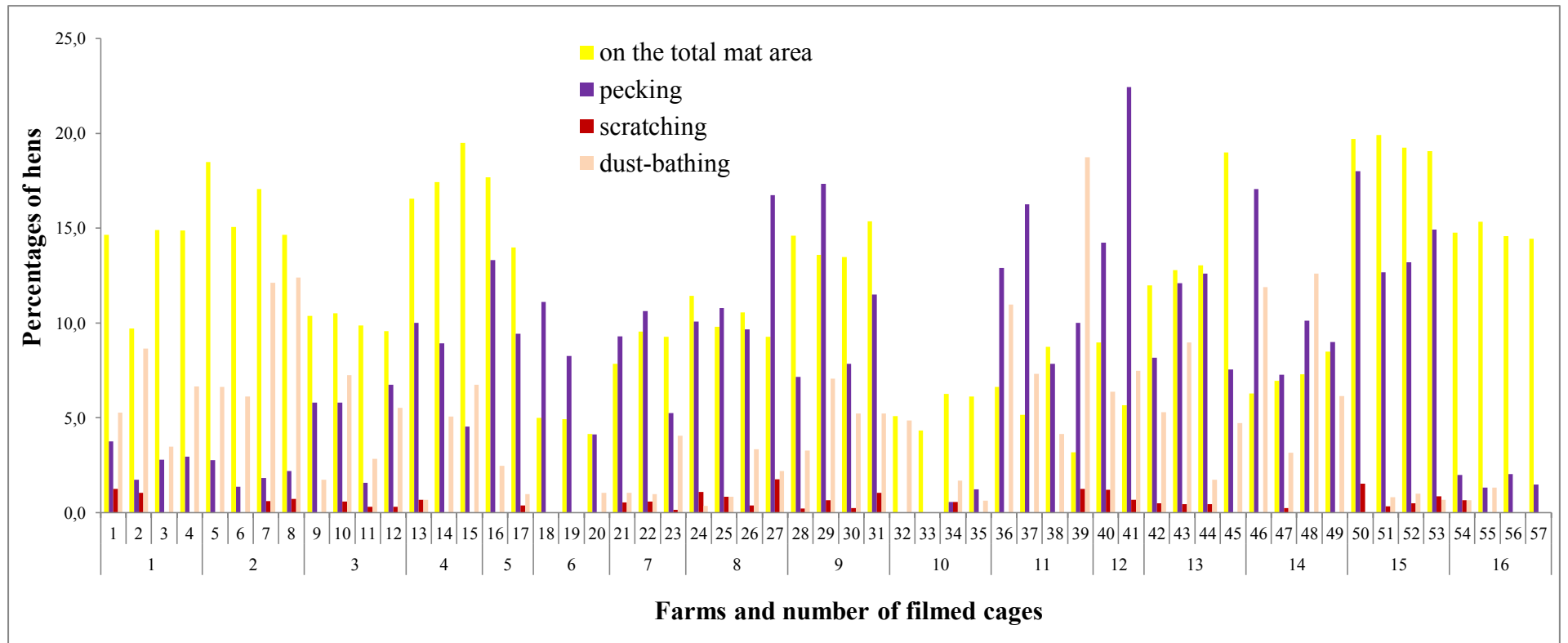


Figure 4.5: Percentages of hens on total mat area, and percentages of hens pecking, scratching and dust-bathing on the observed scratching mats (n = 57) distributed to the 16 farms

Table 4.6: Results of analyses of variance concerning influencing factors on percentages of hens on total mat area, pecking, scratching and dust-bathing with farm set as random factor (n = 57, df = 48)

	% of hens on the total mat area			% pecking		
Fixed effects	Descriptor	p	F	Descriptor	p	F
Intercept	-26.95 ± 9.53	-	-	-17.27 ± 13.52	-	-
Substrate availability	6.53 ± 1.94	0.0015	11.28	3.98 ± 2.75	0.1541	2.10
Number of mats per cage	0.86 ± 0.83	0.3013	1.09	-4.39 ± 1.16	0.0004	14.28
Mat width (cm)	-0.05 ± 0.05	0.3340	0.95	-0.03 ± 0.07	0.6827	0.17
Mat area per hen (sqcm)	0.06 ± 0.04	0.1173	2.54	0.12 ± 0.06	0.0360	4.66
Cage stocking density (hens/sqm)	2.67 ± 0.68	0.0003	15.38	2.49 ± 0.97	0.0133	6.61
Group size (number of hens/cage)	-0.01 ± 0.05	0.8881	0.02	-0.13 ± 0.07	0.0573	3.79
Light intensity (Lux)	0.94 ± 0.35	0.0103	7.13	0.58 ± 0.42	0.1727	1.92
	% scratching			% dust-bathing		
Fixed effects	Descriptor	p	F	Descriptor	p	F
Intercept	-3.13 ± 1.40	-	-	3.45 ± 11.72	-	-
Substrate availability	0.12 ± 0.29	0.6847	0.17	8.05 ± 2.38	0.0015	11.42
Number of mats per cage	-0.07 ± 0.12	0.5546	0.35	-0.79 ± 1.01	0.4351	0.62
Mat width (cm)	-0.01 ± 0.01	0.1389	2.26	0.15 ± 0.06	0.0174	6.06
Mat area per hen (sqcm)	0.01 ± 0.01	0.0461	4.19	-0.08 ± 0.05	0.0851	3.09
Cage stocking density (hens/sqm)	0.21 ± 0.10	0.0456	4.21	0.41 ± 0.84	0.6248	0.24
Group size (no. of hens/cage)	0.01 ± 0.01	0.0505	4.02	-0.09 ± 0.06	0.1148	2.58
Light intensity (lx)	-0.01 ± 0.04	0.8868	0.02	-0.53 ± 0.36	0.1514	2.13

Significance level: p = 0.05

4.4 Discussion

4.4.1 Behaviours

Feasibility of the behavioural observations was good, with in most cases identifiable behaviour ($92 \pm 5\%$). Thum (2009) reached similar values (95%) when observing the whole cage area in similar cage systems. Intra- and inter-observer reliability was acceptable to very good, although for scratching behaviour no testing was possible because of non-occurrence in the test videos. Indeed, scratching was performed by the average hen only during $0.4 \pm 0.5\%$ of the observation time. Though some caution may be advisable when interpreting the scratching data due to the lacking reliability testing, the good intra- and inter-observer reliability regarding scratching behaviour in the observations in the aviary systems with the same observers (Chapter 3, Table 3.5) increases trust in the data recorded.

Calculated on the basis of number of hens on the observed mats, presumably $12 \pm 5\%$ of the hens were on average present on the total mat area. This is on a similar level as results found in similar cage systems by Rönchen et al. (2010, 8 ± 1 and $10 \pm 5\%$ of hens from two layer strains), Telle (2011, 9 to 19 %) and Thum (2009, 14 %) or LayWel (2006) with a much wider range of 5 to 20 % of all hens on the litter areas in small furnished cages. Sewerin (2002) found even only 4 % of the hens on the mat in the small furnished cage 'Aviplus'. In the visited hen houses the total mat area corresponded to on average 9 % of the whole cage area. This means that slightly more hens used the mats than to be expected by chance. As the percentages of hens performing specifically the behaviours the mats should allow or stimulate were relatively low, it can be hypothesized that hens preferred to stay on the mats also for other reasons such as a more comfortable flooring, or that the available mat area was too low to allow these space-consuming behaviours for a sufficient number of hens (see further discussion of this below). As a note of caution it must be mentioned that due to different positions of the mats within the cages the number of hens on the filmed mats were not necessarily the same than on the other mats in the cage. Different proximity to other structures in the cage or differing light intensities may have influenced the presence of hens on the various mats and by this biased results. However, if only the number of hens per filmed mat would have been used, the large variation in number of mats and mat area per cage between systems could not have been taken into account which was judged to be clearly more disadvantageous.

On average $8 \pm 5\%$ of hens were performing pecking. This was half the time recorded by Thum (2009), who found 19 % of hens pecking which, however, included object pecking. Possibly this is a reason for the differing results. In fact, Weitzenbuerger (2005) found even only 1 ± 2 and $1 \pm 0\%$ pecking hens on the scratching mats of two different furnished cage systems ('small group housing'). In general, the pecking levels occurring in furnished cages are lower than those recorded on littered areas (25 %, Chapter 3) and much lower than under near natural conditions in semi-wild Red Junglefowl with 60 % of the active part of a day spent with ground pecking (Dawkins 1989). At the same time, the range of pecking behaviour between the observed cages was from 0 to 22 %, so that in several cages no pecking behaviour occurred at all or at a very low level. Scratching was performed to even lower

extent, from 0 to 2 % of the observation time. Compared to semi-natural conditions with hens spending 34 % of the active time ground scratching (Dawkins 1989), generally the amount of scratching behaviour found in commercial hen houses is extremely low. While results demand for improvement of scratching mats so that they better stimulate exploratory behaviour, such improvement may be difficult, because scratching mats were found to be littered only for short time because the substrate is pecked immediately after provision and removed from the mats due to scratching (Sewerin 2002, Weitzenbuerger 2005, Thum 2009).

Dust-bathing was shown by on average 4 ± 4 % of hens on the mats. These findings are much higher compared to previous studies of Rönchen et al. (2010, 0.002 ± 0.10 and 0.5 ± 0.2 % with two different layer strains), Telle (2011, 0.5 ± 1.7 %), Weitzenbuerger (2005, 0.4 ± 0.1 and 0.5 ± 0.1 % in two different systems) and FAL (2005, 1 to 2 % of main activity period in small furnished cages). Thum (2009) found more similar figures of 3 %, or Sewerin (2002) of 6 % on non-littered mats, in contrast to 15 % on littered ones. In comparison to the reference value of 1 to 2 %, calculated on the basis of 20 to 35 minutes dustbathing every second day (Fölsch 1981, Vestergaard 1982, Van Liere et al. 1990) distributed over a 16 hours light day, it could be argued that the investigated furnished cage systems meet the hens' requirements for sufficient opportunity to dust-bathe. However, as the time sampling method applied does not give any information about completeness of the individual dust-bathing events, some reservations need to be made. Furthermore, considering the lack of dust-bathing substrate, it is questionable how rewarding the execution of sham-dust-bathing is for the birds. Lindberg and Nicol (1997) investigated the dust-bathing behaviour in laying hens in different modified cage systems, comparing the dust-baths in littered (sand) bust-baths with dust-bath performances on the wired cage floor. The authors concluded that the provision of loose pecking substrate and space for the hens to dust-bathe in a location where sham dust-bathing occurs uninterrupted is acceptable concerning modified cages. On the other hand they mention the addition welfare benefit of an extra dust-bathing facility. Considering that it is the general opinion to increase hen welfare, sham dust-bathing rather may not an alternative to the hens and the effort must be to optimize the conditions of the litter areas, especially due to substrate provision, to provide adequate opportunities for the hens in terms of litter directed behaviours. Possibly the partly relatively high amount of time the hens spent dust-bathing was caused by longer dust-baths in which hens tried to compensate for the lack of adequate substrate. In addition, the large range in time spent dust-bathing from 0 to 19 % shows that not all houses provided conditions that allowed sufficient dust-bathing in quantitative terms.

4.4.2 Substrate availability

Only on nine farms littered scratching mats were found. This is a hint that mats on most farms were not littered regularly or that substrate was even never provided, which was the case on two farms, where the feeder pipe was blocked. The significance of substrate availability was confirmed in the present study. It affected the percentages of hens on the total mat area and the amount of dust-bathing (8 % more). Also Sewerin (2002) found similar increases when scratching mats were littered, in this case with non-food material. In contrast, the very low percentages of dust-bathing found by Rönchen et al. (2010) and Telle (2011) were observed on mats which were littered regularly, even four times daily with 35 g feed each in the latter

study. Furthermore, Hergt (2007) did not find any significant differences in the frequency of dust-bathing between non-littered and littered (35 g feed four times a day) Astroturf with on average 27 ± 3 and 32 ± 2 dust-bathing bouts, respectively. Although this does not preclude that the duration of dust-bathing may have been different, it is also possible that hens used the feed rather for foraging behaviours than for dust-bathing. This may also depend on the dimensions of the mats and the mat area available per hen (see below). Furthermore, the characteristics of the mats themselves may matter. Weitzenbuerger (2005) concluded that artificial grass mats allow better to manipulate the material because the particles fall between the stalks. Telle (2011) found less dust-bathing behaviour towards the end of the laying period, presumably due to a high abrasion of the turf. In the present study, most farms (13) had artificial grass mats which all showed signs of abrasion, but only affecting small areas of the mats. In general, it is very likely that factors such as these play a role in the explanation of the differences between studies concerning the effect of substrate on mats on the amount of dust-bathing.

Another aspect is the necessary amount of substrate to be effective. Up to date no information is available on this question. Sewerin (2002) and Weitzenbuerger (2005) assumed that often substrate is provided only in the mornings as well as in too little amounts, leading to decreased dust-bathing. Additionally, the type of substrate, feed or different non-feed materials, may be important. Unfortunately, no variation in substrate type was found on the visited farms and the amount of substrate could not be measured because it was not provided while the scientists worked in the hen house. Thus, these questions remain open for further investigations.

Contrary to expectations, no influence of substrate availability on exploratory behaviour could be detected. This is in line with the findings of Weitzenbuerger (2005) concerning sawdust who concluded that sawdust granules are not appropriate to stimulate pecking and scratching behaviour in furnished cages. In contrast, Hergt (2007) found significantly more pecks, though no more scratching, on mats littered with feed compared to non-littered conditions. It is easily perceivable that feed is more stimulating to peck than sawdust. However, concerning the own results, apparently the hens used the mats littered with feed rather for their dust-bathing than for pecking and scratching behaviour. At the same time scratching occurred at very low level and over a low range, which could be the reason that no influencing factors could be detected. Additionally, it is not certain that substrate actually was present during the video observations, because the assessment of substrate provision took place at minimum two days before the video observations started.

4.4.3 Number of mats per cage, mat width and mat area per hen

Mat number and width differed between the farms, and consequently also size per mat. In general, a lower mat number meant that larger mats were used. These factors are specific for the individual systems (Telle 2011). The wide range concerning total mat area per cage (713 to 6,650 sqcm) certainly had to do with the large range in cage and group size between farms. However, the total mat area of on average 82 sqcm was not in compliance with the legislative requirements.

The percentage of hens pecking decreased with increasing mat number about 4 % per mat. At the same time increasing mat area per hen led to more hens pecking. Both results taken together may mean that larger litter areas of one piece may be more appropriate to enable ground pecking. Furthermore, possibly more consumable particles were available for every single hen with increasing mat area per animal.

Secondly, dust-bathing behaviour was positively affected by increasing mat width. This conforms to the results from Telle (2011) who observed longer dust-baths on wider mats compared to conditions with several smaller ones. Appleby et al. (1993) found hens not being able to turn around or stretch completely during their dust-bath with a dust-bath size of 600 sqcm per test cage for four hens. They concluded that the provided space was too low to perform complete dust-baths. Lindberg and Nicol (1997) came to the same conclusion that dust-baths in conventional and modified cages are relatively narrow and less suitable, because hens rotate and change sides during dust-bathing. In rectangular dust-baths the authors observed apparently restricted sideways leg and rubbing movements, although the actual sizes of the investigated dust-baths were not mentioned.

Thirdly, scratching behaviour increased with more mat area provided for every single hen. Dawkins and Hardie (1989) observed that Ross brown hens covered 655 to 1,217 sqcm of the litter area while ground scratching.

All of these results indicate that enhancing the size of the scratching mat and area per hen will contribute to increased exploration and dust-bathing behaviour.

Size and area of the mats did not significantly influence the percentages of hens on the total mat area. Possibly, the more hens are stimulated to express space-consuming exploration and dust-bathing behaviour on the mats, the less birds fit onto one mat. As already discussed above, apparently the hens are attracted to stay on the mats for different purposes. However, as the mat area provided per hen on the visited farms was extremely low, it can be expected that the possibility for each hen to stay on a mat for these different purposes was very limited. It would be interesting to compare the present conditions with conditions including a much higher provision of mat space per hen.

4.4.4 Cage stocking density and group size

Cage stocking density is regulated by the TierSchNutzV (2006) with a minimum space allowance of 800 sqcm per hen with < 2 kg body weight (900 sqcm > 2 kg body weight). Compared to the allowed 12.5 hens/sqm the recorded stocking density regarding the number of hens at the start of lay was sometimes too high (at maximum 14 hens/sqm), but on average below the legal limit.

Data regarding group size were taken from the farm managers' information about the number of hens housed at the beginning of the laying period. These numbers may have decreased due to mortality, but were unfortunately not ascertained specifically for the filmed cages. Therefore, it is possible that group size and stocking density were partly and to differing degrees lower than assumed, which may have biased results. Consequently, they should be cautiously interpreted.

Higher cage stocking densities were positively associated with the percentages of hens on the total mat area, pecking and scratching. While it appears plausible that under conditions of

decreased space allowances, more hens have to stay on the mat area, it is amazing that also more pecking and especially scratching were performed for which certain space demands exist. Probably hens were additionally motivated by other hens to perform exploratory behaviour due to social facilitation (Martin 1985).

Group size had no significant effect on any investigated parameter, but there was a tendency that with increasing group size pecking decreased ($p = 0.0573$) and scratching increased ($p = 0.0505$). The latter would be in line with results from Reiter and Bessei (1999) for boiler chickens, but for pecking this is not the case. It can only be speculated that hens changed from pecking to scratching behaviour with more hens per group again due to social facilitation, or that feed particles at the turf surface were already consumed due to more hens within one cage and therefore hens were motivated to use their claws to get particles out between the stalks of the turf mats.

Dust-bathing behaviour was neither influenced by group size nor by stocking density. In contrast, Carmichael et al. (1999) found less dust-bathing with increasing stocking density in laying hens in a perchery system, and Reiter and Bessei (1999) in broilers. In these cases stocking density was directly affecting available litter area whereas in the investigated furnished cages stocking density was not correlated with mat area per hen. Additionally, Reiter and Bessei (1999) found a facilitating effect of group size on dust-bathing which cannot completely be excluded here, as it is possible that hens performed dust-baths more often but with shorter duration. Due to the time sampling method applied, no differentiation between frequency and duration of individual dust-baths can be made.

4.4.5 Light intensity

Light intensity on the filmed scratching mats was on average 1.8 ± 1.9 lx. Thum (2009) found similar light levels of 1.3 to 2.9 lx at the scratching mats. Telle (2001) measured 3.9 to 5 lx on average in the whole cages. With a range of 0 to 8.3 lx some houses did not provide lightened scratching areas. Prescott and Wathes (1999) assumed that single hens on-farm may receive light intensities of less than 2 or on contrast more than 200 lx, depending on their cage position.

The light intensity had significant positive impact on the percentages of hens observable on the mats, with 1 % more hens observable with every increasing lx. However, neither exploratory nor dust-bathing was affected. In general, light intensity was at such a low stage that active behaviours will have been suppressed. Manser (1996) recommends light intensities of 20 lx at minimum to prevent inactivation. Further, Davis et al. (1999) found hens to prefer highest light intensity for active behaviours and lowest (6 lx) for resting. Especially dust-bathing behaviour can be elicited by light (Fölsch 1981, Vestergaard et al. 1990). Buchenauer (2005) observed sham dust-bathing on wired floor in caged hens near light sources, and concluded that scratching mats were not lightened enough to give hens the motivation to dust-bath there. Consequently, the low light levels will have contributed to the hens using the mats for other behaviours of lower activity such as resting. Therefore, illumination of scratching areas on-farm should generally be increased.

4.5 Conclusion

Clear effects of substrate provision, light intensity, scratching mat area per hen and mat size have been identified on the attractiveness of the scratching mats and the exploratory and dust-bathing behaviour performed on them. While it appears that under the investigated commercial conditions on average sufficient opportunity for dust-bathing behaviour is given in quantitative terms, it is questionable whether this also applies to the functional aspects of dust-bathing and to which degree complete dust-baths are occurring. A more detailed analysis of the individual dust-bathing sequences would be helpful in this regard. In addition, the large range in time spent dust-bathing shows that not all houses provided conditions that allowed sufficient dust-bathing in quantitative terms. Exploratory behaviour was only minimally carried out on the scratching mats, likely due to space limitations and the lack of stimulation. Hence, in order to allow hens in furnished cages more normal activity, it can be recommended to improve substrate availability, and to increase mat space and light intensity.

4.6 Literature

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5. The influence of litter management on the qualitative performance of dust-bathing behaviour in laying hens kept in aviary systems

5.1 Introduction

Dust-bathing behaviour belongs to the inherent natural behaviour repertoire in laying hens. It is one of the most complex behaviours (Fölsch 1981) and starts to occur with single elements already in the first week of life (Kruijt 1964). After five weeks dust-bathing is developed in complete order (Kruijt 1964, Williams and Strungis 1979). Possible functions are to maintain the plumage in good condition, to remove feather lipids and dandruffs (Healy and Thomas 1973, Van Liere and Bokma 1987, Van Liere 1992a, 1992b, Olsson and Keeling 2005). It is performed in three steps including several elements: first, suitable substrate is accumulated and then applied to the plumage, secondly, the material is distributed within the feathers and on the skin through rubbing, followed by a resting phase and at last the substrate is removed by axial body shaking (Kruijt 1964, Vestergaard et al. 1990, Van Liere 1992b). On average, a hen kept in floor systems or under near natural conditions performs a dust-bath every second day, lasting about 20 to 35 minutes, mostly at about midday, and in artificial light condition eight hours after light turned on (Fölsch 1981, Vestergaard 1982, Van Liere et al. 1990). Depending on time and conditions, the birds show high motivation to perform this behaviour (Lindberg and Nicol 1997). It is still open, if dust-bathing can be considered as a 'need' in laying hens (Duncan 1998) and if it affects hens' welfare (Olsson and Keeling 2005). Many studies are available on this theme, and results are partly contradictory and often difficult to interpret (reviewed by Olsson and Keeling 2005). Following results from Vestergaard et al. (1997) a lacking opportunity of dust-bath performance may lead to stress. At least compared to cages, hens' welfare appears to be enhanced in aviaries in terms of dust-bathing behaviour (Colson et al. 2007). However, the extent of improvement depends on the detailed conditions in the aviary.

In this context litter is a very important resource (Hughes 1976, Fölsch et al. 1986, Gerken 1989). It is generally expected that longer and more complete dust-baths that end with a body shake are performed in more preferred material (Van Liere et al. 1990, Vestergaard et al. 1990). On this basis, it is already well established under experimental conditions that hens prefer friable and fine materials such as sand or peat (Petherick and Duncan 1989, Van Liere et al. 1990, Sanotra et al. 1995, Shields et al. 2004, de Jong et al. 2005, Olsson and Keeling 2005, de Jong et al. 2007) over materials with greater particle size like straw or wood shavings (Sanotra et al. 1995). However, on-farm conditions have not yet been analysed with respect to type of litter material and its' impact on laying hens' dust-bathing performance. Furthermore, while practical recommendations can be found on the litter height, for instance to provide a height of about 10 cm, diminished to 5 cm if external dust-baths of 20 to 25 cm depth are provided (Baumann 2006), and while organic associations like Bioland (2009) require a minimum litter height of 5 cm, no specific legal standards are set at national (TierSchNutzV 2006) or European level (EC Directive 1999). Likewise, only very little scientific evidence is available on this aspect. Moesta et al. (2008) did not find major differences in dust-bathing between litter depths of two and 20 centimetres when deprived

hens became access to the substrate, but more vertical wing shakes were performed on shallow substrate, possibly because it was more difficult to bring substrate into the plumage. Besides warm and dry conditions and the presence of loose soil in natural surroundings, especially light works as a further dust-bathing behaviour eliciting factor (Kruijt 1964, Fölsch 1981, Vestergaard et al. 1990). In laying hen houses often very restricted light conditions are applied to prevent feather pecking and cannibalism and the question arises, if dust-baths shown are in a comparable range to experimental and near natural conditions. Possibly under commercial conditions there is a lack of the stimulus light.

Also stocking density may have an impact on dust-bathing hens, as it is known that frequent disturbances by conspecifics can lead to abandoned dust-baths (Appleby et al. 1993). Thus, it can be hypothesized that higher stocking densities in the litter areas may lead to shorter and incomplete dust-baths as well as increased disturbances.

The goal of the present study was to investigate under commercial conditions the possible impact of litter material and height, light intensity and stocking density in the litter area on the qualitative performance of dust-bathing, video recorded in the litter areas of 17 commercial German laying hen farms. Based on these results, recommendations for farmers should be elaborated in order to improve on-farm conditions with respect to the opportunity to perform normal dust-bathing behaviour.

5.2 Animals, materials and methods

The current study including 17 commercial aviaries is part of a project on the development of management recommendations for furnished cage and aviary systems, carried out on German laying hen farms. Hens were kept in 15 tier- and two portal-systems (Chapter 3 Figure 3.2) and were at observation time in the last third of their laying period. Flock sizes ranged from 900 to 19,500 hens per house and 400 to 5225 per pen, stocking densities from 6.1 to 18.1 hens per sqm usable area. Aviaries did not contain specific dust-baths, but five did have winter gardens, which were not included into this study because some were not accessible for the hens, for some light intensity measures were lacking and the consequently low number of complete observations under the specific conditions.. Altogether six flocks comprised Lohmann Brown hens, eight Lohmann Brown and Lohmann Tradition (in one case both strains were kept together in one pen), one Lohmann Tradition and Lohmann Selected Leghorn, one Lohmann Brown and Dekalb Weiß and one Dekalb Weiß and Dekalb Braun; in one pen 16 flocks were beak trimmed.

5.2.1 Video recordings

Always 1 sqm parts of the litter area were marked by means of two right angled yards sticks and video recorded (detailed description see Chapter 3, Figure 3.2). In every aviary up to six cameras were installed within the litter area. After a first habituation day, recordings from the second and third day were used for behavioural observations. Recordings were saved digitally on external hard drives.

During camera installation light intensity was measured with a Luxmeter in the litter areas in five positions (near the door, in the middle to the left and right and under the aviary). Measurements were conducted in six directions corresponding to the six sides of a virtual cube. First the average values of the six directions and afterwards one average value for each litter area of all farms were calculated. All housings were artificially illuminated so that no diurnal changes in the light intensity were expected. Information about flock size and litter material was collected with the help of a standardised questionnaire from the farmer. Answers were divided into four classes regarding litter type: Missing litter (1), fine materials (sand, sawdust, cellulose pellets, wood shavings, chalk and mixtures of these, 2), fine materials mixed with straw (3) and straw (4). Litter area size was directly measured and the stocking density in the litter area calculated. Further, litter height was measured at every camera position always in the middle of the frame. Litter condition with reference to identifiability and humidity was assessed as explained in Chapter 3.

5.2.2 Behavioural observations

Behavioural observations were carried out with the help of the software ‘The Observer[®] XT 10.5’ (Noldus Information Technology, Wageningen, The Netherlands). Using instantaneous scan sampling (Martin and Bateson 2007) every two minutes over the first 16 minutes of every second light hour, so that one light day was covered within two observation days (see Chapter 3), instances were identified in the video recordings from 37 cameras where dust-bathing took place. Only such dust-baths were then chosen for further behavioural observations which started within the determined 1 sqm litter area and ended within the video picture. A dust-bath was considered to have ended if the hen stood up, in case no further dust-bath elements followed for 30 seconds or axial body shaking was performed (Table 5.2). No reference to observation day or time of day was made because of the low frequency of dust-baths fulfilling these criteria. One to eight dust-baths per camera and altogether 129 dust-baths were observed in detail using continuous focal hen sampling (Martin and Bateson 2007). Numbers of the behavioural elements defined in Table 5.1 and durations of the phases defined in Table 5.2 for every dust-bath observed were recorded. Pecking, scratching, squatting down and bill raking before the first tossing behaviour were not recorded. Side rubbing that occurred during the dust-baths could not consistently be identified due to partly poor video quality and single hens blocking the view. Therefore, it was not separately recorded. Wing and leg stretching was recorded but not further taken into account. The proportion of the tossing phase was calculated as tossing phase duration (minutes)/dust-bath duration (minutes). Further, the frequency of vertical wing shaking or scratching with one leg per tossing phase was calculated as number of vertical wing shakes or scratches/duration of tossing phase (minutes). The number of disturbances during a single dust-bath was categorized into three classes: 0 disturbances, 1 disturbance or ≥ 2 disturbances.

Two observers were involved in the recordings (observer 1: 47 dust-baths, observer 2: 82 dust-baths). Inter-observer reliability was tested concerning total dust-bath and tossing phase duration, number of vertical wing shaking, scratching with one leg and disturbances during the dust-baths using 16 dust-baths distributed over 10 cameras of eight aviaries. Inter-observer testing concerning ‘total number of hens’ is described in Chapter 3.

Table 5.1: Definitions of the recorded dust-bathing elements

Dust-bathing element	Definition
Vertical wing shaking	The hen lies on her breast with fluffed feathers and shows fast vertical wing movements, immediately followed by rhythmic movements of the legs (Severin 2002, modified after Van Liere and Wiepkema 1992)
Scratching with one leg	Hen lies on her ventral-lateral body side with fluffed feathers and scratches repeatedly and fast with her contra-lateral leg (modified after Van Liere and Wiepkema 1992)
Axial body shaking	The hen stands and shakes her body around the long axis of the trunk with fluffed feathers and somewhat lifted wings in fast movements (modified after Moesta et al. 2008, Fölsch 1981)
Dust-bath disturbances	Hen interrupts her dust-bath following external impact (e.g. after sudden movement of flock or being pecked by conspecifics) and stands up

Table 5.2: Definitions of the recorded dust-bathing phases

Parameter	Definition
Total dust-bath duration	The dust-bath starts when the hen sits down, starting the first vertical wing shake or scratching with one leg. It ends when the hen stands up in case no further dust-bath elements follow for 30 seconds (modified after Van Liere and Wiepkema 1992). Behavioural changes (e.g. preening or standing up) of less than 30 seconds within a dust-bath were excluded from the calculation of the dust-bath duration.
Tossing phase duration	The tossing phase starts with the first performed vertical wing shake or scratch with one leg and ends with the last vertical wing shake or scratch with one leg within a dust-bath as defined above

5.2.3 Statistical analysis

Spearman's rank correlation coefficients were calculated to assess inter-observer reliability, and coefficients greater than 0.7 were regarded to reflect an acceptable agreement (Martin and Bateson 2007).

Mixed models were further calculated in SAS 9.2 ('proc mixed' statement, SAS Institute) for the following dependent variables: Total dust-bath duration, proportion of tossing phase, absolute number and frequency of vertical wing shaking per tossing phase. Fixed factors were the litter height (α_i), litter type class (β_j), disturbance class (γ_k), light intensity (δ_l) and stocking density in the litter area (ϵ_m). Farms as well as cameras were set as random factors. Although concerning frequencies of vertical wing shaking no normal distribution of the residuals was given, compliance with mixed models requirements was assumed, as data were

balanced within the model and more than 40 degrees of freedom were given (Donaldson 1968, Lunney 1970). The model was as follows:

$$y_{ijklm} = \mu + \alpha_i + \beta_j + \gamma_k + \delta_l + \varepsilon_m + \theta_{ijklm}$$

y_{ijklm} = Dependent variable

μ = Intercept

θ_{ijklm} = Residuals

The Spearman's rank correlation coefficient was calculated between the total number of disturbances and the total number of hens observed within the 1 sqm observation frame (for definition of this factor see Chapter 3).

Both, vertical wing shaking and scratching with one leg belong to the tossing phase of a dust-bath. To be able to compare the achieved results with reference values from previous studies, where mostly only vertical wing shaking was taken into account, only a mixed model with this dependent variable was calculated. It was then further tested whether scratching with one leg and vertical wing shaking are correlated by applying a Spearman's rank correlation analysis.

5.3 Results

5.3.1 Inter-observer reliability

Inter-observer reliability was acceptable concerning all parameters (Table 5.3).

Table 5.3: Inter-observer reliability between two observers (n=16)

Parameter	r^{Spearman}
Dust-bath duration (min.)	0.962*
Tossing phase duration (min)	0.991*
Number of vertical wing shaking	0.949*
Number of scratching with one leg	0.939*
Number of disturbed dust-baths	0.995*

*Significance level: $p = 0.01$

5.3.2 Overview over data

Litter was on average 4 ± 2 cm high, ranging from 0 to 9 cm (Figure 5.1). The distribution of the analysed dust-bathing events in terms of the four litter type classes and 16 aviaries is presented in Figure 5.2. The average litter heights corresponding to the different litter types were 4.3 cm for missing litter, 2.6 cm for fine material, 4.1 cm for fine material mixed with straw and 5.3 cm for straw. Altogether 12 dust-baths were observed on humid, the remaining 117 on dry litter areas. 57 dust-baths were performed on identifiable litter material, 72 events were on substrate where the original litter material was not identifiable anymore. On average

1 ± 1.5 disturbances per dust-bath occurred, up to nine at maximum (Figure 5.3). Light intensity was on average 14.5 lx with a range from 1.1 to 99.5 lx (Figure 5.4). The stocking density in the litter areas was on average 27 ± 18 hens per sqm with a range from 11 to 73 hens/sqm.

A dust-bath lasted on average 16.6 ± 6.7 minutes with a wide range from 2.6 to 30.9 minutes (Figure 5.5). Nine of the analysed 129 dust-baths were found to be finished without axial body shaking. On average 69 % of the total dust-bath was the tossing phase, with an average duration of 11.5 ± 5.3 minutes (min: 0.7; max: 26.7). The hens performed on average 20.1 ± 7.9 vertical wing shakes (min: 6; max: 47) per dust-bath and 2.14 ± 2.49 wing shakes per minute of the tossing phase (min: 0.6; max: 28.6). Scratches with one leg occurred 28.0 ± 15.8 (min: 2; max: 81) times per dust-bath and 2.6 ± 2.1 (min: 0.6; max: 24.5) times per minute of the tossing phase. Total numbers of vertical wing shaking and scratching with one leg per dust-bath were significantly correlated ($r_{\text{Spearman}} = 0.590$, $p = 0.01$, Figure 5.6). The total number of disturbances were not correlated with the total number of hens observed ($r_{\text{Spearman}} = -0.178$, $p = 0.01$).

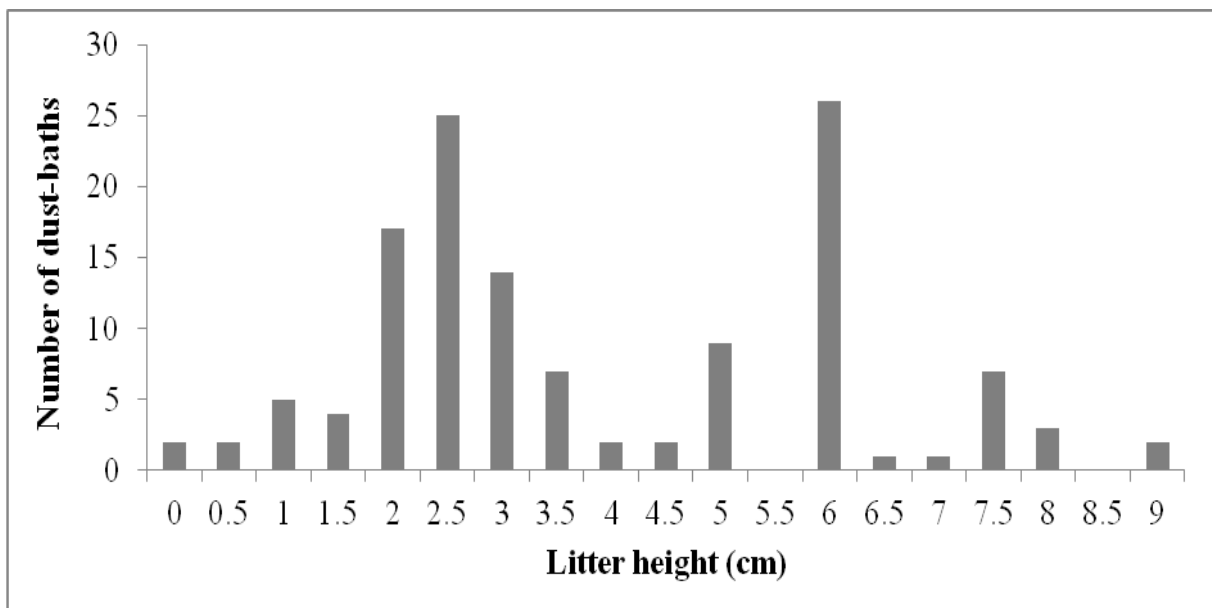


Figure 5.1: Distribution of analysed dust-bathing events (129 in total) in terms of litter height in the respective litter areas

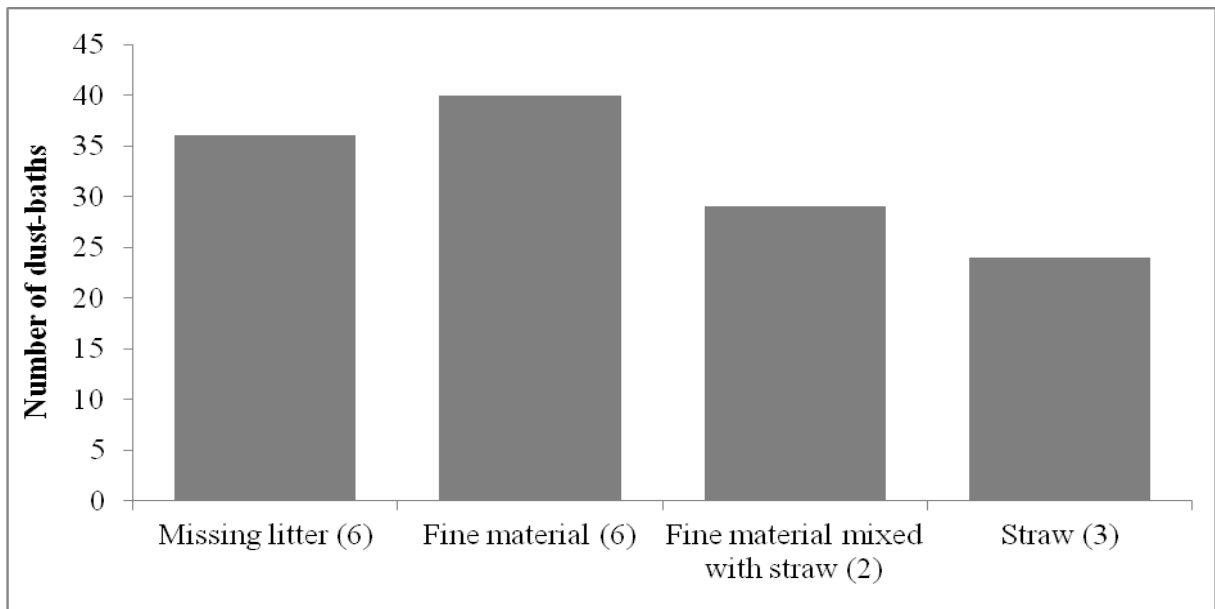


Figure 5.2: Distribution of analysed dust-bathing events (129 in total) with regard to the four litter material classes on which they were performed, number of farms in brackets

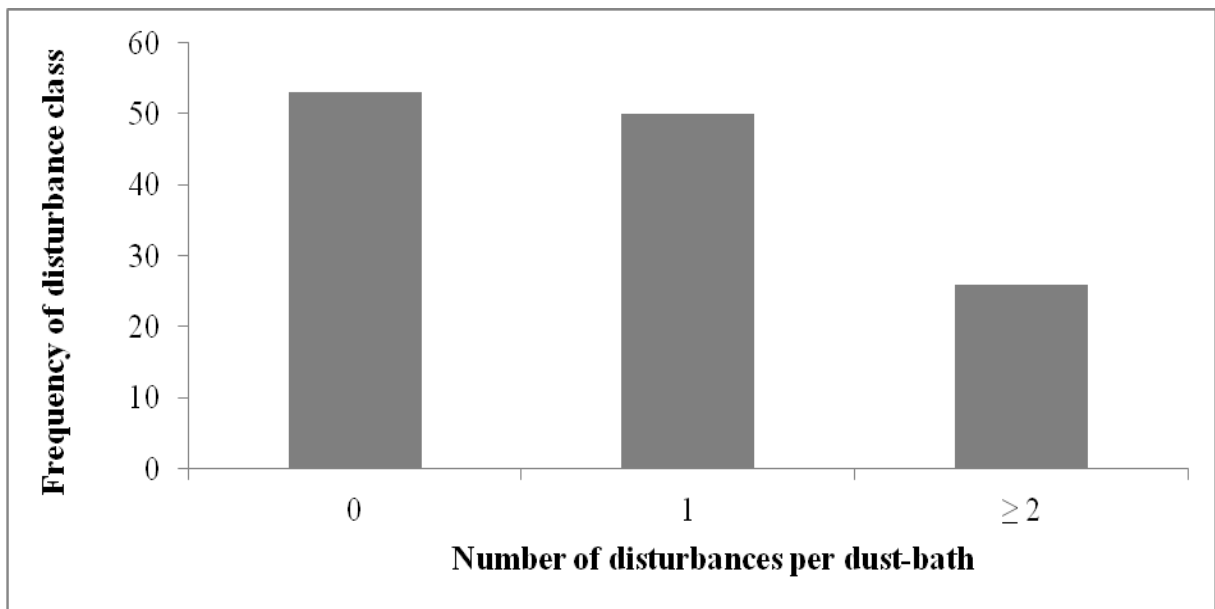


Figure 5.3: Frequency classes concerning disturbances per dust-bath

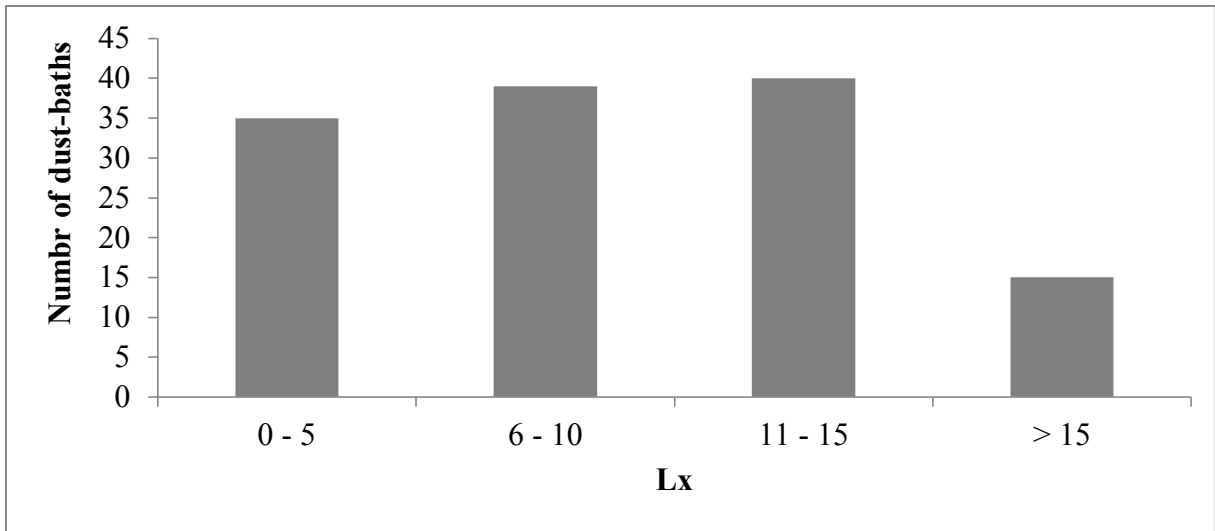


Figure 5.4: Distribution of analysed dust-bathing events (129 in total) with regard to the average light intensity in the litter areas

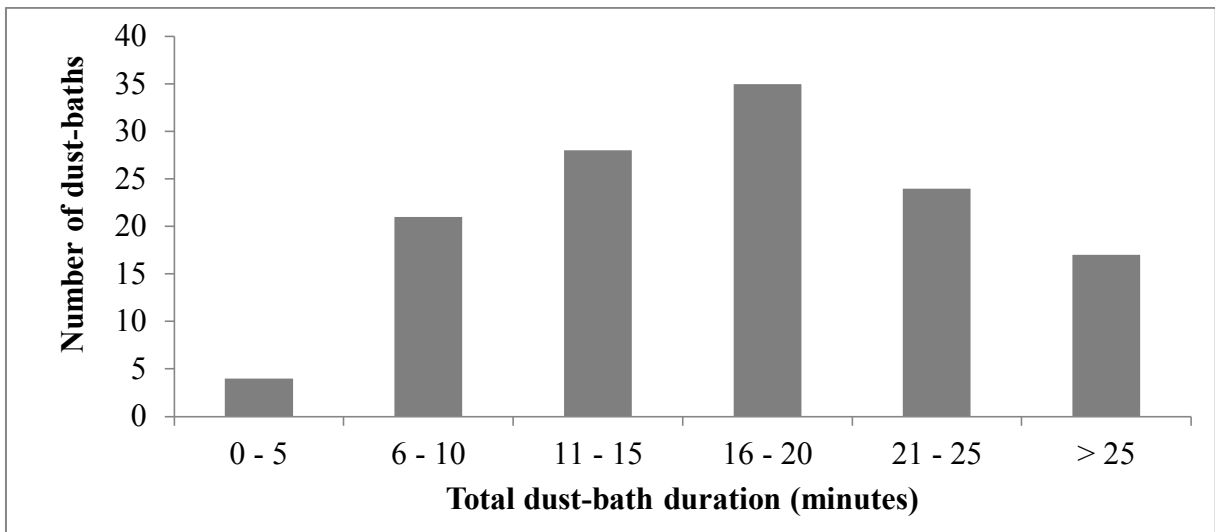


Figure 5.5: Distribution of total dust-bath durations (129 dust-bathing events)

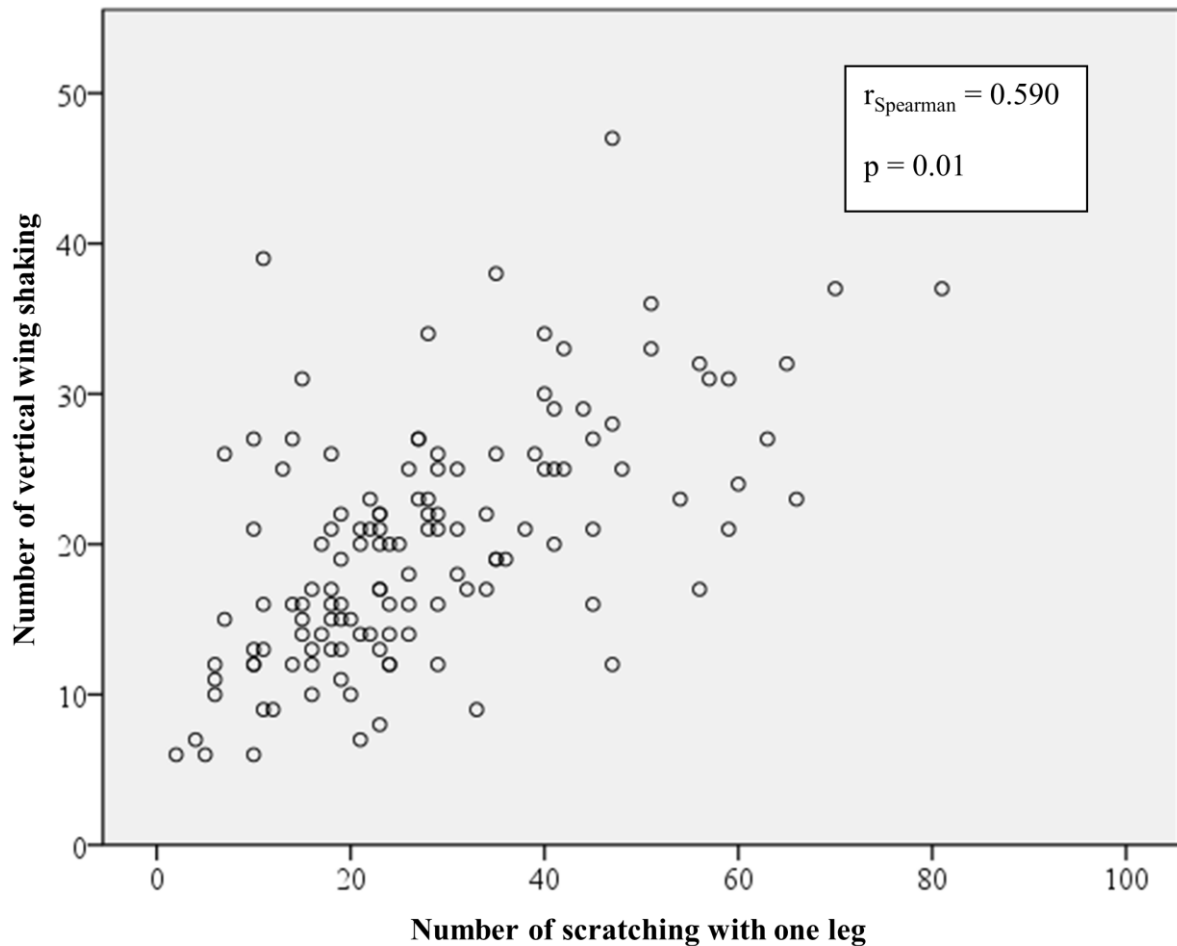


Figure 5.6: Correlation between vertical wing shaking and scratching with one leg, based on 129 dust-bathing events observed

5.3.3 Analyses of variance

With increasing litter height the dust-bath duration decreased significantly (Table 5.4). Litter type class affected all parameters significantly (Table 5.4, least squares means in Figure 5.7): The total dust-bath duration differed between all litter type classes significantly, but no differences occurred between fine material and fine material mixed with straw. The proportion of the tossing phase was significantly smaller on fine litter and fine litter mixed with straw compared to missing litter or pure straw. Vertical wing shaking occurred significantly less often on fine material mixed with straw compared to the other three classes. The frequency of vertical wing shaking per minute tossing phase was found to be highest on fine material compared to the other three classes. The number of disturbances during a single dust-bath had significant impact on the total dust-bath duration, with shortest dust-baths occurring with ≥ 2 disturbances. Furthermore, the frequency of vertical wing shaking was significantly increased with ≥ 2 disturbances (Table 5.4, least squares means in Figure 5.8). With increasing light intensity the proportion of the tossing phase as well as the number of vertical wing shaking significantly decreased. No effect of stocking density in the litter area could be detected (Table 5.4).

Table 5.4: Influencing factors on total dust-bath duration, proportion of the tossing phase, total number of vertical wing shaking per dust-bath and frequency per minute tossing phase (analyses of variance with farm and camera set as random factors, n = 129, df = 118)

	Total dust-bath duration (minutes)			Proportion of the tossing phase (%)			Number of vertical wing shaking			Frequency of vertical wing shaking		
	Descriptor	p	F	Descriptor	p	F	Descriptor	p	F	Descriptor	p	F
Intercept	23.11 ± 2.49	-	-	0.77 ± 0.06	-	-	24.56 ± 3.17	-	-	2.65 ± 1.06	-	-
Litter height (cm)	-0.63 ± 0.28	0.0279	4.96	0.01 ± 0.01	0.4619	0.54	-0.16 ± 0.36	0.6682	0.18	0.02 ± 0.12	0.9005	0.02
Litter type class (4 = intercept)	1 = -5.47 ± 2.02	<.0001	13.22	1 = 0.01 ± 0.05	0.0008	6.00	1 = -2.0 ± 2.53	<.0001	9.07	1 = 0.39 ± 0.85	0.0292	3.11
	2 = -9.65 ± 2.1			2 = -0.12 ± 0.05			2 = -3.59 ± 2.68			2 = 2.03 ± 0.89		
	3 = -11.1 ± 1.98			3 = -0.12 ± 0.05			3 = -10.1 ± 2.46			3 = 0.83 ± 0.82		
	4 = 0			4 = 0			4 = 0			4 = 0		
Disturbance class (≥2 = intercept)	0 = 3.1 ± 1.53	0.0038	5.85	0 = -0.02 ± 0.04	0.15.39	1.90	0 = 2.68 ± 1.96	0.3037	1.20	0 = -1.37 ± 0.66	0.0435	3.22
	1 = 4.87 ± 1.43			1 = 0.06 ± 0.04			1 = 2.68 ± 1.83			1 = -1.51 ± 0.61		
	≥2 = 0			≥2 = 0			≥2 = 0			≥2 = 0		
Light intensity (lx)	-0.02 ± 0.03	0.4121	0.68	-0.001 ± 0.00	0.0030	9.20	-0.08 ± 0.03	0.0161	5.96	0.01 ± 0.01	0.5645	0.33
Stocking density (hens/sqm litter area)	0.02 ± 0.03	0.5865	0.30	0.00 ± 0.00	0.2429	1.38	-0.03 ± 0.04	0.4694	0.53	-0.02 ± 0.02	0.2684	1.24

Litter type classes: 1 = missing litter, 2 = fine material, 3 = fine material mixed with straw, 4 = straw, significance level: p = 0.05

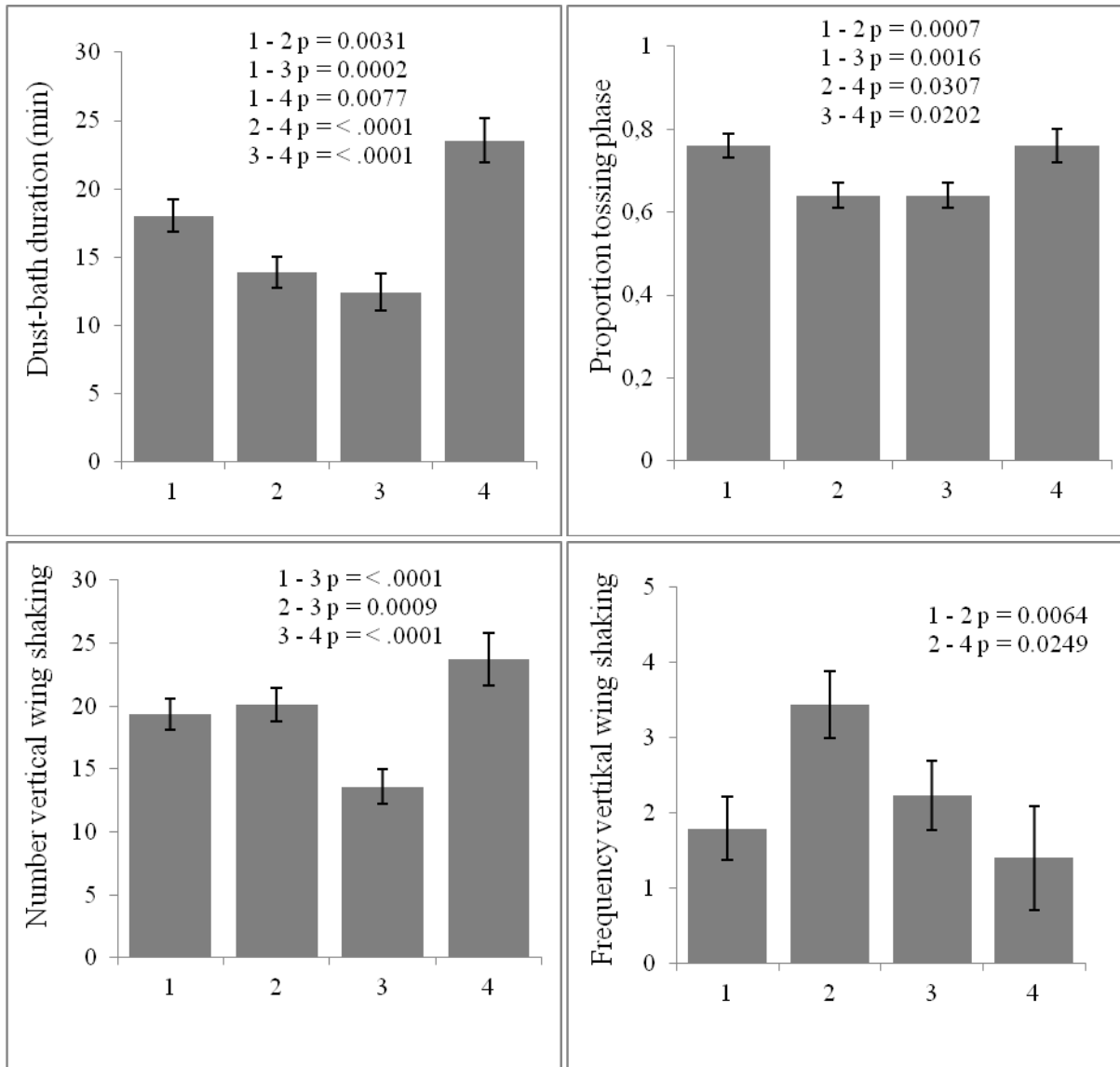


Figure 5.7: Dust-bath duration, proportion of the tossing phase, absolute number of vertical wing shaking per dust-bath and frequency per minute tossing phase corresponding to the four litter type classes (1 = missing litter, 2 = fine materials, 3 = fine materials mixed with straw, 4 = straw; least squares means, standard deviations and p-values of the significant results (analyses of variance, n = 129, df = 118), significance level: p = 0.05

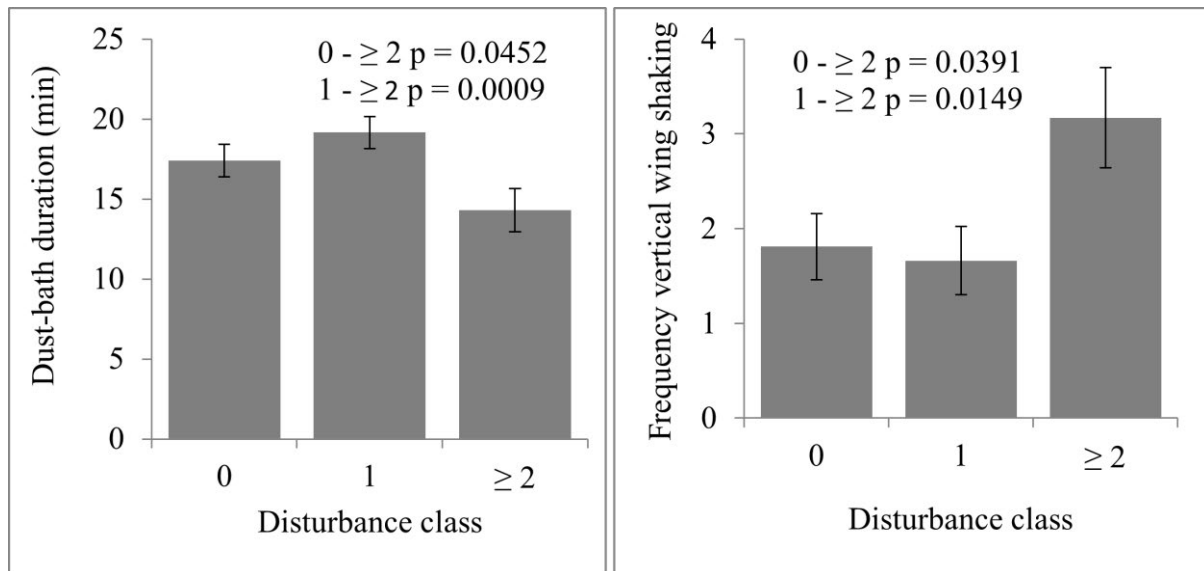


Figure 5.8: Dust-bath duration and frequency of vertical wing shaking per minute tossing phase corresponding to the three dust-bath disturbance classes; least squares means, standard deviations and p-values of the significant results (analyses of variance, $n = 129$, $df = 118$), significance level: $p = 0.05$

5.4 Discussion

5.4.1 Dust-bathing parameters

5.4.1.1 Total dust-bath duration

The average dust-bath duration of about 17 minutes found in the investigated aviaries is somewhat lower than the 20 to 35 minutes reported from previous studies where observations in floor houses and under near-natural conditions took place (Fölsch 1981, Vestergaard 1982, Van Liere et al. 1990). The range between the different dust-baths was from 3 to 31 minutes which is similar to the range Van Liere et al. (1990) observed (5 to 30 minutes) either on sand or wood shavings under experimental conditions. The range between the farms was on average 7 to 23 minutes and the lowest dust-bath durations caused by one farm at one camera position, where five dust-baths with an average duration of 6 minutes were observed. It is supposed that the frequent disturbances at this camera position, ranging from 1 to 6 per dust-bath, caused the short dust-baths. While some of the observed short dust-baths will have been incomplete and have reflected an unsatisfying dust-bathing event for the hen, more than half of the dust-baths (59 %) lasted 20 minutes or more. Furthermore, 93 % of the recorded dust-baths ended with axial body shaking and can therefore be regarded as successful, although axial body shaking may not be a sufficient criterion to assess dust-bath quality, because hens that perform certain tossing behaviour on substrate certainly must remove substrate particles out of their plumage independently of the tossing duration and sequence of the dust-bath. However, based on dust-bath durations and presence of axial body shaking, it can be concluded that in most cases no indication of disturbed dust-bathing behaviour was found.

Still, it must be kept in mind that the selection of analysed dust-baths very likely produced a bias, because only those dust-baths were observed in detail that ended within the observation area, so that dust-baths ending with axial body shaking will be over-represented. This was a problem due to the video recordings covering only a limited area. It happened repeatedly that during dust-bathing hens moved in or out of the video picture which then made it impossible to record the complete event. For the same reason, some very short dust-bath durations may also have been due to hens having already started a dust-bath outside the observation area, which they then interrupted and started again within the frame. Despite this methodical problem, observed dust-baths apparently reflected different conditions on the different farms or in different parts of the litter area as will be discussed below.

5.4.1.2 Proportion of the tossing phase, absolute number of vertical wing shaking and scratching with one leg per dust-bath and frequency per minute tossing phase

On average 69 % of the total dust-bathing time consisted of the tossing phase, with an average duration of about 12 minutes. This relatively large proportion could have been caused by overestimating the tossing phase due to methodical problems. In order to clearly distinguish between the end of the tossing and start of the following resting phase the further parameter 'rubbing' (side laying or side rubbing) was applied in a previous study (Van Liere 1992b), where only about 30 to 50 % of the total dust-bath duration on either sand or wood shavings was the tossing phase. This should have been included into the observation scheme, but partly due to poor video quality and a blocked view of the focal hen caused by conspecifics, it was not possible to consistently differentiate doubtlessly between 'rubbing' and lying on the side during the resting phase. Therefore, the point of change from the tossing phase to the resting phase was set to be the last performance of tossing behaviour (vertical wing shaking or scratching with one leg), and not the performance of rubbing. If hens fell back from the resting into the tossing phase, this time was then allocated to the tossing phase.

A correlation of $r_{\text{Spearman}} = 0.590$ was found between the number of vertical wing shaking and scratching with one leg. This confirms that both elements of the tossing phase of a dust-bath convey similar information. Observed were on average 20 vertical wing shakes, with a frequency of 2 per minute, which was similar to Van Liere (1992b), who also observed 2 vertical wing shakes per minutes of the tossing phase each on either sand or wood shavings in test pens. In contrast, Lindberg and Nicol (1997) found hens to perform vertical wing shaking up to a frequency of 12 and 13 per minute during their dust-bath, corresponding to 129 and 196 vertical wing shakes in total numbers, in with sand filled litter areas of two different modified cages. Moesta et al. (2008) observed on average 48 and 44 vertical wing shakes per dust-bath on either used or fresh wood shavings presented in test pens for caged hens after deprivation of a dust-bath opportunity. It appears that hens adapt their tossing behaviour to the situation in which they get the opportunity to take a dust-bath.

5.4.2 Influencing factors

5.4.2.1 Litter height

An increasing litter height was significantly associated with decreased total dust-bath duration; with every cm more, dust-bathing lasted 0.6 minutes less. Possibly it is easier for the hens to apply litter material into the plumage with a greater height. On the first view, the findings of Moesta et al. (2008) under experimental conditions that hens performed more vertical wing shakes on shallow litter height (2 cm) compared to high litter (20 cm) point into a similar direction. The authors assumed that hens compensated the less suitable litter height by increasing their tossing behaviour. The effect was additionally increased with less suitable litter material (fresh in comparison to used wood shavings containing more fine particles). However, they did not find differences in dust-bathing duration, although this might have been caused by the dust-bathing deprivation of the tested hens. On the other hand, no effects of litter height were found on vertical wing shakes or tossing phase proportion in the present study. Thus, it appears that the reduced dust-bathing duration was due to a shorter resting phase rather than due to reduced tossing behaviour. Currently this cannot convincingly be explained and further investigations on possible effects of litter height on dust-bathing behaviour should be carried out.

5.4.2.2 Litter type

The significantly longest mean dust-bathing duration was found on straw with about 24 minutes whereas with missing litter, on fine material and fine material mixed with straw dust-bathing on average only lasted between 12 and 18 minutes. It is generally expected that longer and more complete dust-baths that end with a body shake are performed in more preferred material (Van Liere et al. 1990, Vestergaard et al. 1990). However, fine and friable material such as sand or peat was found to be preferred by the hens (Petherick and Duncan 1989, Van Liere et al. 1990, Sanotra et al. 1995, Shields et al. 2004, de Jong et al. 2005, Olsson and Keeling 2005, de Jong et al. 2007) over material with greater particle size like straw (Sanotra et al. 1995). It is questionable whether a longer dust-bath duration in any case reflects better dust-bathing conditions and a more rewarding execution of the behaviour. Instead, it is possible that the coarser straw rendered it more difficult to bring the material into the feathers, although the number of vertical wing shakes per dust-bath was only numerically highest, but not significantly different from the classes missing and fine litter. Nevertheless, possible effects of litter type should be taken into account when looking at the usually applied reference values for dust-bathing duration. Vestergaard (1982) observed dust-bathing on deep straw and found average values of 27 minutes and maximum durations of 35 minutes. In contrast Sewerin (2002) observed 20 dust-baths in the free-range area of an aviary house and found average dust-bath duration of about only 20 minutes. Possibly the dust-bath duration assessed in a near-natural surrounding as by Sewerin (2002) is a better reference value for comparison of results achieved under commercial conditions.

With missing litter the second highest dust-bathing duration of 18 minutes was observed, which was significantly higher than on fine material and on fine material mixed with straw. Missing litter meant that the floor was covered by dry dust and faeces in about the same litter heights as the areas littered with fine material mixed with straw. In quantitative terms it appears that this very fine and friable material meets the hens' requirements better than the two other litter types despite the presumably reduced function to remove feather lipids. Dust-bathing on the two other litter types was not only shortest, but also the proportion of the tossing phase was smallest. However, only on fine material mixed with straw this led to a significantly lower number of vertical wing shakes per dust-bath. This was the case, because the frequency of wing shakes per minute tossing phase was significantly increased on fine material. It was numerically second highest on fine material mixed with straw, but differences to the other litter type classes were not significant. Altogether this means that except for the class fine material mixed with straw about the same number of vertical wing shakes were performed in dust-baths of different lengths on the different litter types. Scholz et al. (2010) observed under experimental conditions no significant differences in the number of vertical wing shakes, ranging from 16 to 19 per dust-bath, between food particles, wood shavings and lignocellulose, where dust-bath duration was significantly longer on food particles (18 minutes) compared to the other materials (13 and 12 minutes). Further, Vestergaard et al. (1990) found under experimental conditions with about 25 and 30 vertical wing shakes on either sand or wire mesh no significant differences, but the hens' dust-baths were significantly longer on wire with 37 minutes against sand with 28 minutes. In another experiment the dust-bathing duration differed significantly (30 and 39 minutes) between sand and wood shavings, but not the frequency of vertical wing shaking with each two performances per minute (Van Liere 1992). In modified cages Lindberg and Nicol (1997) observed in dust-baths filled with sand durations of on average 12 to 13 minutes and very short durations on the cage wire of conventional cages (3 minutes). It is not clear whether this reflects a successful adaptation to different litter characteristics or indicates any problems in the shorter or longer dust-baths. It would be necessary to observe longer-term individual dust-bathing behaviour and analyse the degree of soiling of the plumage to better understand the significance of the detailed execution of the dust-bathing behaviour. Likewise, for the class fine material mixed with straw the question remains why less tossing behaviour and shorter dust-baths occurred here. It is possible that certain characteristics of this litter type led to frustration and an earlier stop of the dust-bathing event, but it may also be that it was easier to work substrate into the plumage so that the whole process could be successfully finished earlier. When looking at the proportion of time which the average hen spent dust-bathing over the light day on the different litter types (Chapter 3), the proportion of time was highest on fine litter mixed with straw and pure straw. In case of shorter dust-baths this would mean that they were performed more frequently, which might point at certain problems in the dust-bathing behaviour. Unfortunately in the analysis presented in Chapter 3 the two litter type classes had to be merged because of the small number of farms with these conditions. However, the raw data show that hens performed dust-bathing on average in 12 % of the observation time in the pure straw condition and 9 % on fine material mixed with straw, which is very close together. It can be supposed that certain problems in the dust-bathing behaviour occurred on fine materials mixed with straw.

In general it can be concluded that despite a large body of literature on dust-bathing behaviour some central questions regarding the interpretation of the quantitative and qualitative expression of dust-bathing are still open.

Another aspect of litter quality recorded was whether in the litter area the original litter material was still identifiable. While this may give some indication about frequencies of provision of fresh litter material, it also depends on the chosen material itself, how quickly it degrades during use. For instance, cellulose pellets will degrade much quicker than pure straw. In fact, only the variable litter type was entered into the model, because its explanatory value was higher.

5.4.2.3 Disturbance class

The number of disturbances affected the dust-bath duration, shortest (about 14 minutes) in cases of ≥ 2 disturbances per dust-bath, compared to no disturbances (17 minutes) or one disturbance (19 minutes). Also other studies found that frequent disturbances lead to shorter dust-baths (Lindberg and Nicol 1997, Appleby et al. 1993). De Jong (2005) reported that about 45 % of the dust-bathing events observed in five barn systems under commercial conditions were incomplete, mainly due to pecks from conspecifics towards the dust-bathing hens. In the current study only those disturbances were recorded that caused the hens to interrupt dust-bathing and to stand up. No statement is possible about how pecking on dust-bathing hens that did not lead to an interruption of the behaviour was perceived by the dust-bathing hens, i.e. whether this also affected dust-bathing performance.

Dust-baths were not only shorter when ≥ 2 disturbances occurred, but the frequency of vertical wing shakes per minute tossing phase increased nearly 100 %, so that finally no significant difference in the number of wing shakes per dust-bath was detectable. This is another example of possible adaptation towards external influences, although this does not exclude that disturbances, which occurred up to nine times per dust-bath, partly were at such high level that stress or frustration could have resulted.

As it was expected that the number of disturbances is influenced by the number of birds in the scratching area, the association with the total number of hens observed within the 1 sqm observation frame was analysed. However, no influence could be detected. Possibly the extent of disturbances does more relate to the motivation of single hens to peck on conspecifics, which might also be associated with the flock disposition for feather pecking. Furthermore, part of the disturbances did not originate from individual interactions, but from sudden flock movements that may be independent from the actual number of hens in the observation area.

5.4.2.4 Light intensity

Within the range of light intensities found on the farms (from 1 to 100 lx, on average 15 lx) the proportion of the tossing phase and number of vertical wing shakes per dust-bathing event were reduced with increasing light intensity. With unchanged dust-bathing duration, apparently the resting phase must have been prolonged which is contrary to the expectation that higher light intensity leads to more activity (Manser 1996). However, light as a factor

facilitating dust-bathing (Fölsch 1981, Vestergaard et al. 1990) may cause hens to dust-bathe more often, so that the demand for an intensive tossing phase is less pronounced because the plumage is generally in a good condition. On the first view this assumption is, however, not supported by the results presented in Chapter 3 where no effect of light levels could be detected on the daily proportion of time hens spent dust-bathing. Nevertheless, it must be considered that with instantaneous scan sampling dust-bathing was only recorded when dust-bathing movements were shown. Thus, the extent of dust-bathing may have been underestimated when longer resting phases occurred. A further speculation on possible reasons for the light effect is that a better visual control of the material applied on the feathers may reduce the necessary effort to work the substrate into the plumage.

5.4.2.5 Stocking density

Contrary to expectations, the stocking density of the litter area did not affect any of the assessed parameters, though with a range of 11 to 73 hens per sqm a large variance was found on-farm. However, as discussed above regarding disturbances, possibly other factors played a greater role.

Furthermore, it is known, that hens synchronise their dust-bathing behaviour, which was found to be less distinctive with higher group sizes and stocking densities in broiler chickens (Reiter and Bessei 1999). As a response to higher stocking densities the hens here might have distributed their dust-bathing more evenly over the whole light day, rather than to concentrate it at the middle of the light day as expected from previous studies (e.g. Vestergaard 1982). Additionally, possibly hens were able to adapt their dust-bathing behaviour to different stocking densities by choosing areas with lower hen numbers. It has to be remembered that only a very small part of the whole litter areas was actually observed and no information is available on the total amount of dust-bathing behaviour and quality of dust-baths performed in other areas than analysed here.

5.5 Conclusion

Under the commercial conditions investigated, litter height, litter type and light intensity influenced the execution of the dust-bathing events. Further effects detected were related to disturbances from conspecifics. In general, most observed dust-bathing events (59 %) were found to last 20 minutes or more, and the average duration of about 17 minutes was only moderately lower than that. Keeping in mind that the necessary selection of events due to technical limitations probably led to an over-representation of complete dust-bathings, the results reflect a considerable adaptability within the dust-bathing sequence with respect to durations of the tossing or resting phase and frequency of vertical wing shakes. These adaptations are likely responses to characteristics of the dust-bathing substrate in terms of how easy it can be brought into the plumage, to the stimulation of dust-bathing behaviour and to disturbances. From the data and scientific knowledge available it cannot be judged whether the observed variation reflected successful adaptations or partly frustration in the hens. For this it would be necessary to observe longer-term individual dust-bathing behaviour and to

assess the degree of soiling of the plumage to better understand the significance of the detailed execution of the dust-bathing behaviour, also in functional terms. High numbers of disturbances during dust-bathing should be prevented, but no influencing factors could be identified in the present investigation so that also here further studies are necessary.

5.6 Literature

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6. General conclusions

The on-farm assessment of exploratory and dust-bathing behaviour proved to be challenging, especially in the aviary systems. While camera failure in the cages led to only single cages being not observable, and the video quality was less limiting, in the aviaries more recordings had to be discarded, additionally due to partly high hen numbers in view, dust and mites on the camera lenses, or single hens blocking the view. However, with 57 and 81 cameras analysed in the cages and aviaries, respectively, a moderate sample size could be included into the present work. However, despite using extensive technical video recording equipment, it was not possible to cover a sufficient proportion of characteristic litter areas in the aviaries to record representative data for the individual farms. Instead, it was the aim to capture different conditions within and between farms in selected 1 sqm litter area parts, and to analyse possible effects of these conditions on the hens' exploratory and dust-bathing behaviour. This approach did not allow comparing between different farms, but between the single conditions assessed. The observation of the scratching mats in the cage systems proved to be less difficult, because only very limited areas and fewer hen numbers had to be observed.

Regarding the furnished cages it can be recommended to improve substrate availability, to increase the size of the scratching areas as well as to raise light intensity. Also with regard to the aviary systems litter availability should be improved by providing litter material at the start of and repeatedly during the laying period. Litter height should not be too low.

However, quite a number of open questions remain which require future investigations. For instance, further experimental studies should be carried out to determine litter heights and litter types that better stimulate exploration behaviour. With regard to dust-bathing behaviour observations of longer-term individual behaviour and recording of the degree of plumage soiling or feather lipid content should help to better understand the meaning of the detailed execution of the dust-bathing behaviour.

In general, the environmental conditions on the investigated commercial farms with regard to litter and light were predominantly restrictive in terms of stimuli for exploration and dust-bathing behaviour. Thus, it was only possible to analyse possible associations between these factors and exploration and dust-bathing behaviour within the range found. It would be interesting to be able to include into such a study a much wider range of environmental conditions. Furthermore, possible associations with feather pecking and cannibalism should be investigated to receive more insights into the significance of exploration and dust-bathing behaviour.

7. Summary

The aim of the present thesis was to identify management factors that affect the extent of exploratory behaviour (pecking and scratching) as well as quantitative and qualitative aspects of dust-bathing behaviour in laying hens kept in commercial furnished cages ('small group housing') and aviaries. Based on the results, it should be considered which management measures can be recommended for laying hen farmers in order to enhance hen welfare or in which areas further research is needed.

First in a pilot study, carried out in two commercial aviaries, the applicability of direct observations of dust-bathing behaviour as well as video observations of exploratory (scratching, pecking, searching) and dust-bathing behaviour were tested. The direct observations posed problems of apparent observer influence on the hens' behaviour and regarding feasibility of observations under conditions of high stocking density. Therefore, direct observations were judged to be unfeasible under the commercial conditions encountered. For the analysis of the video recordings, different sampling intervals for instantaneous scan sampling, different extents of observation time, and observations from the same and from different observers (intra- and inter-observer reliability) were compared using correlation analysis, and the most appropriate observation scheme was selected. This consisted of a sample interval of two minutes, applied every first 16 minutes of an hour and distributed over two consecutive light days with on each day every second light hour to be observed. Intra-observer reliability concerning searching behaviour was found not to be sufficient ($r_{\text{Pearson}} \leq 0.7$), and it was consequently excluded from further analyses. Also inter-observer reliability for scratching, pecking as well as dust-bathing behaviour was below the limit ($r_{\text{Pearson}} \leq 0.7$), showing that further training of observers and refinement of definitions was necessary.

Applying the described observation method, the extent of exploratory and dust-bathing behaviour under different environmental conditions was recorded and analysed in 22 aviary systems. With up to six cameras per farm litter area parts of each 1 sqm were video recorded. Altogether recordings from 81 cameras could be analysed. Intra- and inter-observer reliability was tested again with acceptable results ($r_{\text{Spearman}} = 0.71$ to 1). Within the range of environmental conditions found on-farm, pecking, scratching and dust-bathing behaviour was performed on average 25 %, 2 % and 7 % of the observation time. Hen numbers in the 1 sqm litter areas were positively associated with stocking density ($p = 0.0016$) and group size ($p = 0.0178$). More scratching was performed with increasing litter height ($p = 0.0002$) as well as in humid litter areas ($p = 0.0088$). In altogether 28 observation areas (eight aviaries) no litter had been provided, corresponding to a reduced proportion of dust-bathing hens ($p = 0.0453$). No influencing factor on the amount of pecking behaviour could be identified, although a large range from 4 to 60 % was observed. Further investigations are therefore needed to determine significant factors influencing pecking behaviour as well as appropriate litter heights and litter types that better stimulate exploration behaviour.

The same methodical approach was then used in 16 furnished cage systems. In always four cages per farm the scratching mats were filmed with one camera each and altogether 57 cameras could be analysed. Intra- and inter-observer reliability was tested and found to be sufficient ($r_{\text{Spearman}} = 0.73$ to 1). Scratching did not occur in the test videos, and therefore

could not be tested. With on average three hens on the mats (corresponding to 12 % of the total hen number in the cages) hens spent 8 % of the observed time pecking at the mat, 4 % dust-bathing and 0.4 % scratching. Hence, exploratory behaviour was only minimally carried out on the scratching mats, likely due to space limitations and the lack of stimulation. Higher proportions of the hens in a cage were found on the mats ($p = 0.0015$) and more dust-bathing behaviour ($p = 0.0015$), if substrate was provided. More hens were also on the mats with increasing light intensity ($p = 0.0103$) and increased stocking density ($p = 0.0003$). The latter was also positively associated with the time they spent pecking ($p = 0.0133$) and scratching ($p = 0.0456$). Less pecking occurred with an increasing mat number per cage ($p = 0.0004$), and with increasing mat area per hen the birds spent more time on pecking ($p = 0.0360$) and scratching ($p = 0.0461$). Further, wider mats led to an increased dust-bathing behaviour ($p = 0.0174$). The extent of dust-bathing behaviour observed in the furnished cages was considerably lower than in the aviaries. Whether this reflects a welfare problem cannot be judged on the basis of the current data and available literature. Further, the functional aspects of dust-bathing and to which degree complete dust-baths are occurring should be taken into account, requiring a more detailed analysis of individual dust-bathing sequences and an assessment of plumage condition.

Finally, 129 dust-bathing events recorded in 17 aviaries with altogether 37 cameras, covering each a 1 sqm litter area, were analysed in more detail using continuous focal hen sampling. Inter-observer reliability was acceptable for all assessed parameters ($r_{\text{Pearson}} = 0.94$ to 1). Most observed dust-bathing events (59 %) took 20 minutes or more. On average they lasted 17 minutes, with the tossing phase taking 69 % of this time and including on average 2 vertical wing shakes and 3 scratches with one leg per minute tossing phase. On average 20 vertical wing shakes and 28 scratches with one leg were observed per dust-bathing event. Dust-bathing duration decreased with increasing litter height ($p = 0.0279$). Litter type (categorized as missing litter, fine material, fine material mixed with straw or pure straw) influenced all assessed parameters: dust-bathing duration ($p < 0.0001$), proportion of the tossing phase ($p = 0.0008$), number of vertical wing shakes during the dust-bath ($p < 0.0001$) and frequency of vertical wing shaking per minute tossing phase ($p = 0.0292$). Except for the class fine material mixed with straw about the same number of vertical wing shakes were performed in dust-baths of different lengths on the different litter types. On fine material mixed with straw less tossing behaviour and shorter dust-baths occurred. Furthermore, two or more disturbances of the dust-bathing hens were associated with lower dust-bath duration (0.0038). With increasing light intensity a decreased proportion of the tossing phase ($p = 0.003$) as well as a reduced number of vertical wing shakes ($p = 0.0161$) were recorded. While the necessary selection of events due to technical limitations probably led to an over-representation of complete dust-bathings, the results reflect a considerable adaptability within the dust-bathing sequence with respect to durations of the tossing or resting phase and frequency of vertical wing shakes. These adaptations are likely responses to characteristics of the dust-bathing substrate in terms of how easy it can be brought into the plumage, to the stimulation of dust-bathing behaviour and to disturbances. From the data and scientific knowledge available it cannot be judged whether the observed variation reflected successful adaptations or partly frustration in the hens. For this it would be necessary to observe longer-term individual dust-bathing behaviour

and to assess the degree of soiling of the plumage to better understand the significance of the detailed execution of the dust-bathing behaviour, also in functional terms.

In general, the environmental conditions on the investigated commercial farms with regard to litter and light were predominantly restrictive in terms of stimuli for exploration and dust-bathing behaviour. Thus, it was only possible to analyse possible associations between these factors and exploration and dust-bathing behaviour within the range found. Based on the results the following management recommendations can be given: In order to allow hens in furnished cages more normal activity, substrate availability should be improved and mat space and light intensity increased. With regard to the aviary systems the recommendation is as well to improve litter availability by providing litter material at the start of and repeatedly during the laying period. Litter height should not be too low. High numbers of disturbances during dust-bathing should be prevented, but no influencing factors could be identified in the present investigation so that further studies are necessary.

8. Zusammenfassung

Das Ziel der vorliegenden Arbeit war die Identifikation von Managementfaktoren, welche das Ausmaß des Explorationsverhaltens (picken und scharren) sowie quantitative und qualitative Aspekte des Staubbadeverhaltens von Legehennen, die in kommerziellen ausgestalteten Käfigen ('Kleingruppenhaltung') und Volieren gehalten werden, beeinflussen. Basierend auf den erzielten Ergebnissen sollten Managementmaßnahmen erarbeitet werden, die Landwirten zur Verbesserung des Wohlergehens ihrer Legehennen empfohlen werden können. Des Weiteren sollte herausgestellt werden, in welchen Bereichen weiterer Forschungsbedarf besteht.

Zuerst wurde in einer Pilotstudie die Anwendbarkeit von Direktbeobachtungen zur Erfassung des Staubbadeverhaltens sowie videogestützter Verhaltensbeobachtungen zur Analyse des Explorations- (scharren, picken, suchen) und Staubbadeverhaltens in zwei kommerziellen Volierenställen untersucht. Bezüglich der Direktbeobachtungen ergaben sich Probleme durch einen offensichtlichen Beobachtereinfluss auf das Verhalten der Hennen sowie der Durchführbarkeit der Beobachtungen unter den Bedingungen hoher Besatzdichten. Unter den vorgefundenen kommerziellen Bedingungen wurden daher die Direktbeobachtungen als nicht durchführbar angesehen. Zur Auswertung der Videoaufnahmen wurden verschiedene sampling intervals für das instantaneous scan sampling, unterschiedliche Beobachtungszeiten und Beobachtungen von den selben sowie von unterschiedlichen Beobachtern (Intra- und Interobserver Reliabilität) mittels Korrelationsanalysen getestet. Ausgewählt wurde das am besten geeignete Beobachtungsschema. Dieses sah die Auswertung der jeweils ersten 16 Minuten einer Stunde, verteilt auf zwei konsekutive Lichttage, mit einem sample interval von zwei Minuten vor. Bezüglich der Intraobserver Reliabilität stellte sich das Suchverhalten als nicht reliabel erfassbar heraus ($r_{\text{Pearson}} \leq 0.7$) und wurde aus weiteren Analysen ausgeschlossen. Für die Interobserver Reliabilität konnten bezüglich des Scharr-, Pick- und Staubbadeverhaltens keine ausreichende Übereinstimmungen erlangt werden ($r_{\text{Pearson}} \leq 0.7$), sodass weiteres Training sowie eine Verfeinerung der Definitionen nötig waren.

Die in der Pilotstudie erarbeiteten Methoden der videogestützten Verhaltensanalyse kamen in 22 Voliersystemen zur Untersuchung des Ausmaßes des Explorations- und Staubbadeverhaltens unter verschiedenen Umgebungsbedingungen zur Anwendung. Mit bis zu sechs Kameras pro Betrieb wurden 1 m² große Areale der Scharrräume aufgenommen und insgesamt 81 Kameras ausgewertet. Für die erfassten Verhaltensparameter wurden Intra- sowie Interobserver Abgleiche mit ausreichend hoher Übereinstimmung erzielt ($r_{\text{Spearman}} = 0.71$ bis 1). In der Spannweite der untersuchten Umgebungsbedingungen auf den Betrieben wurde das Pick- und Staubbadeverhalten der Hennen in der Einstreu durchschnittlich mit 25 und 7 % der beobachteten Zeit ausgeführt, bezüglich des Scharrens wurden jedoch nur durchschnittlich 2 % der Zeit genutzt. Als positive Einflussfaktoren auf die Anzahl der Hennen in den untersuchten 1 m² großen Arealen konnte die Besatzdichte ($p = 0.0016$) sowie die Gruppengröße ($p = 0.0178$) ermittelt werden. Bezüglich des Scharrverhaltens wurde dies in höherer Einstreu ($p = 0.0002$) sowie auf feuchten Arealen ($p = 0.0088$) vermehrt ausgeführt. Insgesamt 28 Beobachtungsareale (acht Ställe) boten den Hennen keine Einstreu, was sich in einem verminderten Anteil staubbadender Hennen äußerte (0.0453). Das Pickverhalten wurde von keinen der gewählten Faktoren beeinflusst, obwohl eine große

Spannweite von 4 bis 60 % beobachtet wurde. Somit sind weitere Untersuchungen notwendig, um bedeutende Einflussfaktoren auf das Pickverhalten sowie angemessene Einstreuhöhen und -materialien zu ermitteln, welche das Explorationsverhalten in einem höheren Maß fördern.

Dieselbe Methode wurde nachfolgend in 16 Kleingruppenställen angewendet. In jeweils vier Käfigen pro Stall wurde jeweils eine Scharrmatte mit einer Kamera gefilmt. Insgesamt konnten 57 Kameras ausgewertet werden. Für die Intra- und Interobserver Reliabilität wurden ausreichend hohe Übereinstimmungen erzielt ($r_{\text{Spearman}} = 0.73$ bis 1). Jedoch trat in den Testvideos kein Scharrverhalten auf, welches folglich nicht getestet werden konnte. Mit durchschnittlich drei Tieren auf den Matten (entsprechend 12 % der Gesamttierzahl in den Käfigen) beschäftigten sich die Tiere zu 8 % der Beobachtungszeit mit picken, zu 0.4 % mit scharren und zu 4 % mit staubbaden. Möglicherweise führten die Hennen Explorationsverhalten aufgrund von eingeschränkten Platzverhältnissen sowie mangelnder Stimulation zu einem nur so geringen Anteil aus. Positiv wurde der Anteil der Hennen auf den Matten ($p = 0.0015$) sowie der Zeitanteil am Staubbaden ($p = 0.0015$) von dem Vorhandensein von Substrat sowie ersterer Parameter von einer höheren Lichtintensität ($p = 0.0103$) beeinflusst. Während mit einer höheren Mattenzahl in den Käfigen weniger Pickverhalten beobachtet wurde ($p = 0.0004$), hatte eine erhöhte Mattenfläche je Henne einen positiven Effekt auf das Pick- (0.0360) und Scharrverhalten ($p = 0.0461$). Des Weiteren wurde auf breiteren Matten mehr Staubbaden gezeigt ($p = 0.0174$). Mit einer höheren Besatzdichte erhöhte sich der Anteil Tiere auf den Matten ($p = 0.0003$) sowie der Zeitanteil den die Tiere mit picken ($p = 0.0133$) und scharren ($p = 0.0456$) verbrachten. Das Staubbadeverhalten wurde in den Käfigen im Vergleich zu den Volieren zu einem deutlich geringeren Ausmaß ausgeführt. Inwiefern dies ein Problem bezüglich des Wohlergehens der Hennen bedeutet, kann anhand der vorliegenden Ergebnisse sowie der vorhandenen Literatur nicht beurteilt werden. Des Weiteren sollten funktionale Aspekte des Staubbadeverhaltens sowie der Anteil an vollständig ausgeführten Staubbädern in Form von Gefiederbeurteilungen sowie Detailauswertungen zur Abfolge individueller Staubbadevorgänge mit in Betracht gezogen werden.

Abschließend wurden 129 Staubbadevorgänge aus 17 Volierenställen, verteilt auf 37 Kameras, die jeweils 1 m² große Scharraumareale filmten, detaillierter mittels kontinuierlicher Fokustierbeobachtungen untersucht. Die Interobserver Reliabilität war für alle erfassten Verhaltensparameter akzeptabel $r_{\text{Pearson}} = 0.94$ und 1. Der Großteil an beobachteter Staubbadevorgänge (59 %) dauerte länger als 20 Minuten. Die durchschnittliche Staubbadedauer lag bei 17 Minuten und setzte sich zu 69 % aus der Aufbringphase zusammen, in der die Tiere pro Minute 2 vertikale Flügelschläge sowie 3 Mal Scharren mit einem Bein ausführten. Die absolute Anzahl vertikaler Flügelschläge betrug durchschnittlich 20, Scharren mit einem Bein wurde durchschnittlich 28 Mal beobachtet. Mit einer höheren Einstreu wurde eine geringere Staubbadedauer ermittelt ($p = 0.0279$). Die Art der Einstreu (unterteilt in fehlende Einstreu, feine Einstreu, feine Einstreu gemischt mit Stroh oder reines Stroh) hatte einen Einfluss auf alle erhobenen Parameter: Staubbadedauer ($p = < .0001$), Anteil der Aufbringphase ($p = 0.0008$), Anzahl der vertikalen Flügelschläge während des gesamten Staubbades ($p = < .0001$) und Frequenz der vertikalen Flügelschläge pro Minute der Aufbringphase ($p = 0.0292$). Außer bezüglich der Einstreuart 'feine Einstreu gemischt mit Stroh' wurde etwa die gleiche Anzahl vertikaler Flügelschläge während der Staubbäder

unterschiedlicher Länge auf den verschiedenen Einstreuarten ausgeführt. Auf feiner Einstreu gemischt mit Stroh wurden weniger Aufbringverhalten sowie kürzere Staubbäder beobachtet. Des Weiteren waren zwei oder mehr Störungen während eines Staubbades mit einer verkürzten Staubbadedauer assoziiert ($p = 0.0038$). Eine Erhöhung der Lichtintensität bewirkte einen geringeren Anteil der Aufbringphase ($p = 0.003$) sowie eine reduzierte Anzahl vertikaler Flügelschläge ($p = 0.0161$). Aufgrund von technischen Einschränkungen war es nötig eine Auswahl an auswertbaren Staubbadevorgängen zu treffen, was wahrscheinlich zu einer Überpräsentation von vollständigen Staubbadevorgängen geführt hat. Die Ergebnisse spiegeln eine beträchtliche Anpassungsfähigkeit innerhalb der Staubbadesequenzen bezüglich der Zeitdauern von Aufbring- und Ruhephase sowie der Frequenz des vertikalen Flügelschlagens wider. Diese Anpassungen resultieren vermutlich aus den Charakteristika der Staubbadesubstrate, die unterschiedlich leicht in das Gefieder eingebracht werden können, der Stimulation des Staubbadeverhaltens sowie der Störungen. Anhand der vorliegenden Daten und vorhandenen wissenschaftlichen Erkenntnissen ist es jedoch nicht möglich zu beurteilen, ob die beobachtete Variation eine erfolgreiche Anpassung oder teilweise Frustration der Hennen wiedergibt. Hier wäre es nötig, längerfristig individuelle Staubbäder zu beobachten sowie den Ölgehalt des Gefieders zu beurteilen, um die Bedeutung der Ausübung des Staubbadeverhaltens im Detail besser zu verstehen sowie dessen funktionaler Aspekt.

Insgesamt gesehen sind bezüglich der Stimulation des Explorations- und Staubbadeverhaltens die Umgebungsbedingungen der untersuchten landwirtschaftlichen Betriebe in Hinblick auf Einstreu und Licht überwiegend eingeschränkt. Es war nur möglich, in der gefundenen Bandbreite mögliche Zusammenhänge zwischen diesen Faktoren und dem Explorations- und Staubbadeverhalten zu untersuchen. Basierend auf den erzielten Ergebnissen können folgende Managementempfehlungen gegeben werden: Um den Hennen in ausgestalteten Käfigen eine bessere Möglichkeit zu geben, ihre normale Aktivität zu zeigen, sollte die Substratverfügbarkeit verbessert sowie die Mattenfläche und die Lichtintensität erhöht werden. In Bezug auf die Volieren wird ebenfalls empfohlen, die Substratverfügbarkeit zu verbessern, indem den Hennen bereits zu Beginn und wiederholt während der Legeperiode Substrat angeboten wird. Die Einstreuhöhe sollte nicht zu niedrig sein. Eine hohe Anzahl von Störungen während des Staubbadens sollte vermieden werden, jedoch konnten in der vorliegenden Untersuchung keine Einflussfaktoren identifiziert werden, sodass weitere Untersuchungen nötig sind.

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Susanne Döring