# FUNCTION AND ORGANIZATION OF DUSTBATHING IN LAYING HENS

**Voor de kippen**

**Promotor:** **dr. P.R. Wiepkema**
**hoogleraar in de ethologie**

**D.W. van Liere**

# FUNCTION AND ORGANIZATION OF DUSTBATHING IN LAYING HENS

**Proefschrift**

ter verkrijging van de graad van doctor
in de landbouw- en milieuwetenschappen
op gezag van de rector magnificus,
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**Liere, D.W. van, 1991. Function and organization of dustbathing in laying hens (Functie en organisatie van stofbadgedrag bij leghennen).** Dustbathing in laying hens (*Gallus gallus domesticus*) serves to remove excessive feather lipids which accumulate and become stale during dust deprivation. In addition and probably as a consequence of lipid removal the fluffiness of the downy feather parts is enhanced. A dustbath consists of appetitive tossings and consummatory rubbings. Its function as well as its organization depend on the nature of the bathing litter. The uninterrupted performance of rubbing is crucial and predicts consistent bathing litter preferences. An increase in stale feather lipids enhances the tendency to bathe, while sham-dustbathing occurs during dust deprivation. However, during long-term deprivation sham-dustbathing develops abnormally. This seems due to intrinsic reinforcement. Long-term deprivation of functional stimulation prescribed by phylogenetical standards may result in an uncontrollable motivation to dustbathe. *Ph.D. thesis, Department of Animal Husbandry, Ethology Section, Agricultural University, P.O. Box 338, 6700 AH Wageningen, The Netherlands.*

# Stellingen

"Scientists have to welcome reductionism as a method, because we can learn an immense amount even from unsuccessful or incomplete attempts at reduction; problems that are left open this way belong to the most valuable possesions of science. The number of interesting and unexpected results we may acquire on the way to our failure can be of the greatest value."

Popper K.R., 1974. Scientific reduction and the essential incompleteness of all science. In: Studies in the philosophy of biology (Editors: F.J. Ayala and T. Dobzhansky), Macmillan Press Ltd., London, pp. 259-284.

1. Stofbadgedrag van kippen is functioneel in het verwijderen van overtollig en mogelijk verschaald verenvet.
   Dit proefschrift.

2. In tegenstelling tot Vestergaard et al.'s conclusie, dat de organisatie van schijnstofbaden zich normaal ontwikkelt, lijkt de uitvoering van dit schijngedrag vooral het finale zijwrijfgedrag te faciliteren.
   Vestergaard, K., Hogan, J.A. & Kruijt, J.P., 1990. Behaviour 112: 99-116.
   Dit proefschrift.

3. Stille wrijvingen hebben diepe gronden.
   Dit proefschrift.

4. De verklaring waarom bepaalde gedragselementen eerder dan andere als afwijkend gedrag geritualiseerd raken heeft een diersoortoverstijgende waarde voor fundamentele ideeën ten aanzien van de organisatie van gedrag en voor de beoordeling van de kwaliteit van de dierhouderij.

5. Het onderzoek aan pikgedrag van kuikens, dat in het kader van de "nature-nurture" problematiek gedaan is, heeft een grote betekenis voor het begrijpen van verenpikgedrag.
   Dawkins, R., 1968. Z. Tierpsychol., 25: 170-186.
   Hogan, J.A., 1971. Behaviour, 39: 128-201.
   Hogan, J.A., 1973. J. Comp. Physiol. Psychol., 83: 355-366.

6. Het gegeven dat mensen, afhankelijk van ervaring, intenties van andere Vertebraten kunnen schatten, lijkt in proximate zowel als ultimate zin nuttig. Dit vermogen bevordert het rendement van het gedragsonderzoek aan dieren.

7. Een verkorting van de universitaire studie vereist des te meer dat de betrokken docenten onderwijskundig geschoold zijn.

8. Alleen als aangenomen wordt dat dieren mentale belevingen kennen, is het begrip "dierlijk welzijn" ethisch zinvol.

9. Het is eerder terecht dierwelzijnsvraagstukken voor te leggen aan psycho-neurobiologische generalisten dan aan veterinaire of ethologische specialisten.

10. Bij toepassing van non-parametrische statistiek is het in de ethologie algemeen geaccepteerde gebruik van een rekenkundig gemiddelde als beschrijvende parameter minder gepast.

cf. Nowak, R., 1991. Anim. Behav., 42: 357-366.
Orgeur, P., 1991. Appl. Anim. Behav. Sci., 31: 83-90.
Sales, G.D., 1991. Behav. Proc., 24: 83-93.
Ziporyn, T. & McClintock, M.K., 1991. Behaviour, 118: 26-41.

11. De standaardisatie van proefdierpopulaties zoals die ter vermindering van het aantal proefdieren voorgestaan wordt, verwijdert de proefdierkundige van de essentie te verklaren waarom er eigenlijk variatie is.

van Zutphen, L.F.M., Baumans, V. & Beynen, A.C., 1991. Proefdieren en dierproeven. Bunge, Utrecht, 365 p.

12. Het buiten beschouwing laten van gedragsobservaties, die intuïtief wel, maar strikt genomen niet binnen de randvoorwaardes van de operationele gedragsdefinitie vallen, illustreert in een notedop de beperkte waarde van de natuurwetenschappelijke ethologie voor de werkelijkheid.

13. Erik weet binnen het raamwerk van het schilderij "Wollewei", dat het eigenlijke leven erbuiten begint; eenmaal daar blijkt hij echter wereldvreemd. Een wetenschapper kan als Erik zijn.

Bomans G., 1951. Erik of het klein insectenboek. Het Spectrum, Utrecht, 208 p.

Stellingen behorend bij het proefschrift

# Function and organization of dustbathing in laying hens

D.W. van Liere, 10 december 1991, Wageningen.

# VOORWOORD

Dit proefschrift omvat onderzoek aan en gedachten omtrent stofbadgedrag bij kippen. Het onderzoek is razend interessant en leerzaam geweest, maar is nog lang niet voltooid. De gedachten zijn nog lang niet uitgerijpt. Het heeft me keer op keer gefrappeerd hoeveel je van dieren over jezelf kunt leren. Mensen zetten zichzelf op een hoger plan, maar in hoeverre is dat terecht gezien de duidelijke homologieën tussen mensen en, bijvoorbeeld, kippen? Het zoeken naar een antwoord gemodelleerd aan onderzoek naar het hoe en waarom van stofbadgedrag is voor mij een belangrijke drijfveer in het promotiewerk geweest.

Met name met Wilma heb ik veel van gedachten kunnen wisselen over het werk in het algemeen en het onderzoek in het bijzonder. Vanuit haar professionele ervaring met humane gedragstrategieën en haar fundamentele interesse in dieren is ze een hele goede en kritische luisteraar geweest, die me stimuleerde zelf kritisch te zijn en twijfels te relativeren. Ellen, onze dochter, heeft me regelmatig met mijn gedachten uit het onderzoek getrokken en op de "normale wereld" gezet. Dat was nodig, omdat het erop vastbijten tot erop stuklopen kon verworden en waar blijft dan de humor, meneer Sonneberg? Wat de humor en steun betreft wil ik zeker Hans Romberg bedanken. Hé, balletje!, was een normale begroeting en typeert voor insiders de nodige meligheid. Hans was er, maar eigenlijk had je d'r geen moer aan. (Je moet natuurlijk weer uitkijken met dit soort opmerkingen: outsiders, het tegenovergestelde wordt bedoeld). Met het personeel van de pluimveeaccomodatie "de Haar" liep de samenwerking gesmeerd. Met name Aad Rodenburg heeft nogal wat verzorgingswerk voor me verzet. Zijn betrokkenheid met het werk en de praterij, bijvoorbeeld over Joegoslavië, heb ik ten zeerste gewaardeerd. Van de studenten, die bij het onderzoek betrokken zijn geweest wil ik Judith Ben Michael noemen. De discussies waren zeer nuttig, bovendien heeft de samenwerking met haar me gesterkt in het idee, dat in het leerproces tijdens zoiets als een stage, de begeleider tussentijds oordelen gerelateerd aan het einddoel moet weten te formuleren en weten kenbaar te maken. The work, enthusiasm and critical attitude of Natasja Siard as a guest worker from the university of Ljubljana (Slovenia), has been very valuable. Her thorough preparation in ethology, despite the lack of ethological facilities in her country struck me and, for me, proved her high motivation.

Het is voor mij zeker van betekenis dat Kees Zoeter, onderdelen van het proefschrift (General Introduction, General Discussion, Chapter 6 en Summary) taalkundig gecorrigeerd heeft. Wie van ons had dat op de middelbare school kunnen bevroeden. Mijn Engels viel best mee naar ik begreep, maar zijn verduidelijkende final touches..., prachtig gewoon! In dezelfde lijn, maar dan op het Nederlandse vlak, wil ik ook Lidwien van der Pas bedanken voor de doorlichting van de Samenvatting.

Gethin Thomas wil ik speciaal bedanken voor zijn positieve kritieken op het idee, dat lipideregulatie middels stofbaden voor de attractie van parasieten van belang zou kunnen zijn. De bijdrages van Jaco Kooijman en Françoise Wemelsfelder zijn ook belangrijk geweest: elders in dit proefschrift zal dat duidelijk worden. Daarnaast wil ik ze van harte bedanken voor hun activiteiten ter voorbereiding van de promotie.

Grote waardering heb ik voor professor Piet Wiepkema. Zijn aandacht voor het integrale biologische functioneren van het individu, gerelateerd aan het verleden van het individu is voor mij een voorbeeld geweest in het proberen te begrijpen waarom er zoveel variatie bestaat tussen dieren van één soort. Een relativering van de rol, die de statistiek in het onderzoek speelt, geeft een scholing in deze aan. Ik vond het heel prettig, dat hij me "liet gaan", maar wel altijd de deur open had voor een "tussendoor" gesprek, waarin met enkele rake opmerkingen zijnerzijds mijn gedachten geordend werden. Ik hoop van harte dat ons goede contact voortduurt.

Naast de genoemde personen wil ik alle anderen, die in het onderzoek en proefschrift een steen (groot en klein) hebben bijgedragen van harte bedanken. Ik zal dat zeker nog mondeling doen.

Diederik

# CONTENTS

# GENERAL INTRODUCTION

Dustbathing is an intriguing maintenance behaviour, likely to have significant adaptive value, as it is found in numerous species of birds, as well as mammals. As far as birds are concerned, Galliformes bathe in dust only (Simmons 1964), whereas many other species may bathe in water as well (Heinroth 1955). Compared with the latter, fowl can be considered "specialists" in the use of dust, and contrary to other birds fowl do not depend on water for purposes of integumental maintenance. Despite their specialization, dustbathing at first sight does not seem to be organized in a clear way: the patterning differs considerably within as well as between individuals (cf. in quail: Borchelt 1975; in hens: Vestergaard 1982; Huber 1987). However, when the behaviour is observed more closely, a basal structure can be recognized. In hens on sand the first bathing tendencies can be recognized by a variable scratching with two legs, and bill raking in the dust. Gradually, the feathers become erected, and the hen squats down. While lying with fluffed feathers, the most evident element is the vertical wing shake (fig. 1): the hen lies on her breast and scratches both legs over the soil backwards and upwards raising the dust between the trunk and the outwardly held wings onto and between the fluffed feathers. Furthermore, a hen may lie on her side with fluffed feathers and scratch the litter on her body with one leg (scratching with one leg), or she may rub her head with fluffed feathers in the dust (head rubbing, fig. 1). Bill raking, which brings dust closer to the bathing animal, mostly precedes and ends the sequence of scratching with one leg, head rubbing and vertical wing shaking. However, after going through several of these sequences the feathers become flattened. A next phase in dustbathing is initiated, when bill raking precedes side lying and side rubbing. During side lying, a hen lies on her side with flattened feathers and wings held tightly to the body, while during side rubbing she also stretches her leg, causing her to rotate to some extend along the longitudinal axis (fig. 1). In contrast to the earlier mentioned elements, side lying and side rubbing are not very active and conspicuous, but they should not be mistaken for resting behaviour in which the hen mostly lies on her breast, while the postures of the feathers and wings are relaxed. Now and then some bill raking, scratching with one leg, head rubbing or vertical wing shaking may interrupt side lying and side rubbing, but after a certain quantity of the latter behaviour the hen stands up and ends her dustbath. At this moment about 20 minutes have elapsed since vertical wing shaking began, and the hen may fluff her feathers again, while shaking her body and wings vigorously. In this way the dust is removed from the plumage. After standing up from side lying and side rubbing a hen may sometimes start other behaviour which may be performed while the feathers and wings still are kept tightly to the body. Body/wing shaking will mostly follow after a variable amount of time (up to half an hour). In conclusion, a flow of preparatory and assimilating behavioural elements can be recognized in the course of a dustbath, although its performance is highly variable. This may suggest that a hen monitors the effect of a bathing element in detail and finely tunes the subsequent one. This illustrates in what way fowl may be considered dustbath "specialists". A refined organization could be crucial for instance in coping with variable qualities of dust. The dust may vary in humidity, but may also vary in its organic composition, particle size, particle structure etcetera. A thorough understanding of the function of dustbathing and of the interplay between the different dustbathing elements would seem to be essential in predicting what a hen requires of her dustbathing environment.

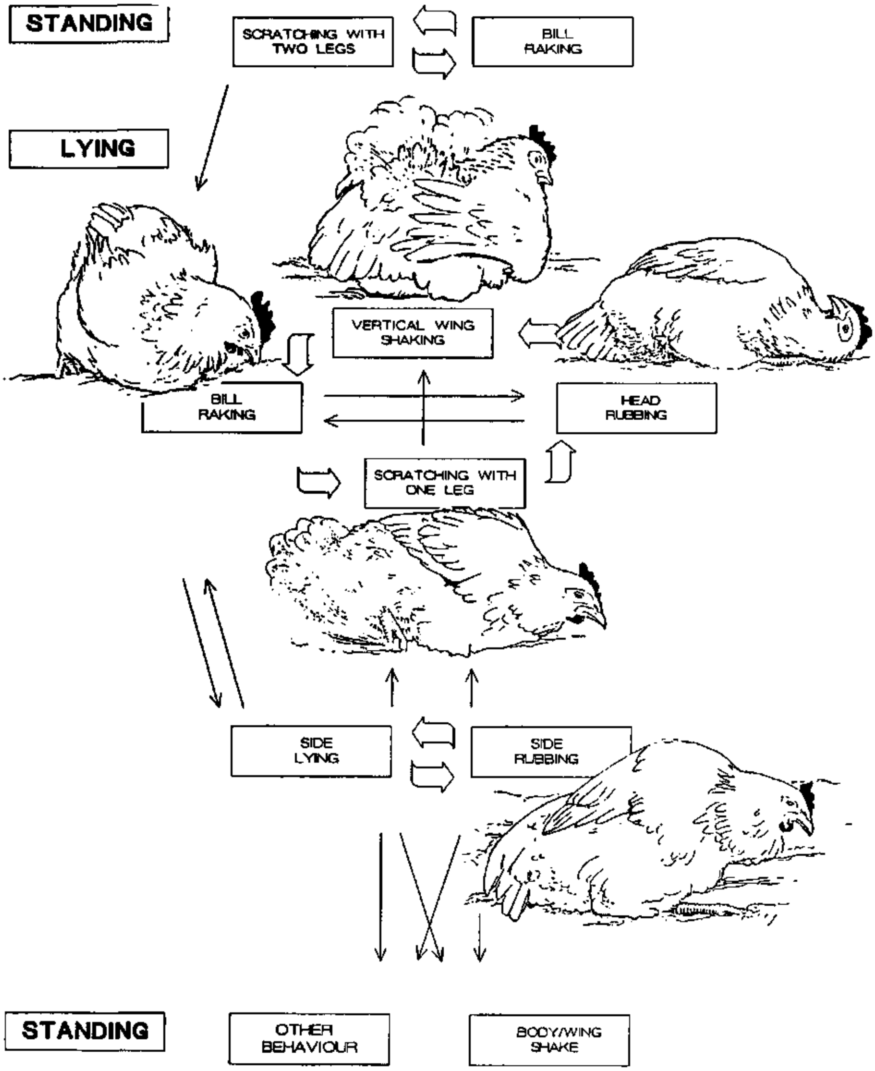

Figure 1. Sequence of complete dustbathing behaviour in laying hens; the width of an arrow is arbitrarily chosen and corresponds to a high or a low transition probability. (Incomplete dustbathing: see Chapter 2).

Given her specialized bathing abilities in dust, how would a hen experience a prolonged and total lack of litter? This condition is not likely to occur in nature, but is a reality for millions of hens kept in intensive husbandries such as battery cages. From a biological and an ethical point of view it is highly relevant to realize that under these circumstances hens keep up dustbathing behaviour (Black & Hughes 1974; Martin 1975; Vestergaard 1980; Bessei & Klinger 1982; Vestergaard et al. 1990). What causes them to perform this sham-activity, how is it organized and what is the use as compared to actual dustbathing? These questions need to be answered to evaluate hens' capacity to adapt their maintenance strategy in a dustless environment: the other side of the coin may be, that fowl are "too specialized" to achieve this. In the latter case the behaviour would not be effective and a discrepancy between the actual and the expected stimulus value (expected as described by Baerends 1976), causing a dustbathing response might remain. From a mechanistic point of view it would be highly interesting to see how a chronic discrepancy would feedback on the organization of the animal's behaviour. However, if it is accepted that a (higher) animal such as a chicken has mental experiences, hens' welfare is at stake too. In the phylogenetic thinking it is appropriate to regard feelings in Vertebrates homologous to those in man, as such an approach corresponds to the one applied in e.g. anatomy, physiology and neurology. In man feelings related to chronic stress are experienced in circumstances which are uncontrollable; in hens this may hold when they are deprived of dust for a long period of time. With respect to animal welfare an international assembly of ethologists ranked the significance of dustbathing behaviour among the ten most urgent subjects for research in the applied field (Wiepkema et al. 1983).

From a cost and benefit point of view it is of interest to point to the mechanism of natural selection, which can be thought to have shaped dustbathing behaviour into being a part of the fowl's behavioural repertoire. With a zootechnical reductionism this mechanism shares the characteristic of omitting redundant and costly features. It may therefore be a mistake to neglect the significance of dustbathing in modern poultry husbandry. Care for dustbathing may be thought superfluous, as the link with the main interests in the poultry industry, survival, growth and egg production, is not clear. However, the fact that there is no clear relation does not mean that there is no significant one; clarity depends on insight in the matter and this may be limited. Therefore, if an energy saving mechanism has been at work in the phylogenetic course of development, it would be of much interest to know in what way dustbathing behaviour is functional. In conclusion, it is biologically, ethically, and zootechnically of much interest to investigate the effect of dustbathing behaviour in hens, as related to its organization in different bathing conditions. The thesis presented here is aimed at supporting this interest. As the experience with dustbathing behaviour in hens has accumulated during the study, the research chapters follow a chronological order.

Chapter 1, 2, 5 and 6 involve the bathing performance in different types of litter and its effects on the plumage. The first chapter deals with the significance of sand for some parameters of dustbathing behaviour and for lipid content and fluffiness of the feathers. The second chapter compares sand and wood-shavings as a bathing litter with regard to preference, bathing performance and the effect on feather lipids in general. The fifth chapter compares in detail the effects bathing in sand and wood-shavings have on the performance of tossing and rubbing, as well as on lipid reduction from proximal and distal feather parts. In the sixth chapter the reinforcing effect of the experience with wood-shavings or sand on the choice of bathing litter, i.e. peat, sand and wood-shavings, is compared with that of a no litter experience. In addition, it is tested whether the earlier found effects, including the effects on plumage surface temperature align with ultimate preferences.

Chapter 3 deals with a causal feature of bathing behaviour which could be relevant in understanding the occurrence of sham-dustbathing, which is the topic of the fourth chapter. In chapter 3 observations of oiling behaviour and their application in testing the causal effect of lipid quantity and lipid quality on subsequent bathing behaviour are considered. Chapter 4 describes how bathing behaviour develops during and after deprivation of sand for more than 5 months. It also deals with the significance of the bathing behaviour as such, by testing the effect of preventing hens to perform sham-dustbathing in particular. Finally, the major findings will be discussed in an integrated approach: aspects of the functionality and the organization of the dustbath are considered within the scope of integumental maintenance, the performance of sham(vacuum)-behaviour, as well as within the scope of litter related effects in poultry husbandries.

## Chapter 1

# SHORT-TERM FEATHER MAINTENANCE AS A FUNCTION OF DUSTBATHING IN LAYING HENS

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## ABSTRACT

Laying hens, deprived of dust for 33 days, showed an average increase in amount of lipids on back feathers from 10.3 to 14.5 mg lipids per g feathers at the end of the dust deprivation period. After the hens could dustbathe again, the original level was restored within 2 days. Also the downy parts of these feathers appeared to be fluffier. Dustbathing presumably regulates the amount of feather lipids and maintains down structure in good condition. These conclusions are discussed in relation to earlier findings concerning feather lipid quantity and feather structure.

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## INTRODUCTION

In Galliformes birds, dustbathing seems to be relevant in feather maintenance (Simmons 1964). During a dustbath, dust is tossed and rubbed between the feathers, which intensifies the contact between dust and feathers. At the end of a dustbath, dust is shaken out of the plumage. Extensive descriptions of dustbathing behaviour are given by Kruijt (1964) and Klinger (1985).

Although many ideas have been put forward about how dustbathing behaviour could contribute to plumage or integument condition, very few have been tested experimentally. Borchelt & Duncan (1974), working with quail, found an accumulation of feather lipids when these birds were deprived of dust. Their results confirmed earlier observations that quail which were deprived of dust appeared greasy (Borchelt et al. 1973; Healy & Thomas 1973). Therefore dustbathing was thought to remove an excess of feather lipids, although supporting evidence was lacking.

It has been suggested that lipids keep feathers supple, which reduces wear and chances of breakage (Elder 1954; Simmons 1964). Since feather lipids oxidize on contact with air and become stale (Simmons 1964), both quantity and quality of feather lipids may affect the function of the plumage.

Dustbathing in quail also removes dandruff and improves feather structure (Healy & Thomas 1973). These conclusions were based on a comparison of feathers collected at the end of a 17 day dustdeprivation period with those collected after birds had had a renewed access to dust. Although only data from pennaceous parts were given, downy parts were also considered. This distinction seems obvious in a functional sense, but is also expressed in the distribution of lipids over the feather itself (Ishida et al. 1973).

Laying hens held in battery cages are unable to bathe in any dusty substrate, which could result in a physical deficiency if dustbathing is functionally important. Therefore the present study aims to investigate the effect of dustbathing on the amount of feather lipids and the structure of both pennaceous and downy feather parts in laying hens.

## MATERIAL AND METHODS

### *Animals and housing*

Twenty beak-trimmed laying hens (Warren) were obtained at the age of 18 weeks from a commercial hatchery. They had been reared on deep litter in a group of about 8000 individuals. At their arrival, the hens were leg ringed for individual identification, randomly divided into 4 groups of 5 hens and kept on deep litter. At the beginning of the experiment, the hens were 10 months of age. Each group was housed in a 150 cm x 240 cm x 260 cm (length x width x height) pen, floored with wooden slats; nest boxes without litter were provided. In a small compartment (measuring 75 cm x 150 cm x 100 cm) adjacent to each pen, a 60 cm x 60 cm x 12 cm dust tray was placed, filled with river sand and refilled once a week. The entrance of the small compartment could be locked by means of a sliding door. The pens in a row were visually isolated from each other and maintained on a 15/9 light dark cycle (lights on at 0500 h). Food from a feeding trough, about 7 cm in diameter, and water were provided ad libitum. Air temperature and relative air humidity were recorded continuously, averaging 20 (*SD* = 4)°C and 74 (*SD* = 12)%, respectively.

### *Experimental design*

The experimental period covered a total of 38 days, divided into a pre-control period (days 1-3), a treatment period (days 4-36) and a post-control period (days 37 and 38). During the pre- and post-control period, all birds had free access to the dust tray. In the treatment period, however, for the first and the third group in the row, the small compartment was locked (the deprived hens), while the other two groups were continuously allowed to dustbathe (the control hens).

### *Feather sampling and analysis: Feather lipids*

From 6 standardized locations on the back of each hen, apparently clean and mature feathers were cut off at their base in equal numbers. This occurred on each day of the pre- and post-control period and the last 3 days of the treatment period. The feathers collected from each hen were grouped together per period. Thus, 3 samples per hen, which weighed 2-3 g and contained about 90 feathers each, were acquired for lipid extraction. Seven days after sampling, feather lipids of each sample were extracted according to the Soxhlett cold extraction method described by the I.S.O. (Anon. 1983). After extraction with diethyl ether for 2 h, the solvent was removed by distillation. The lipid residue was dried for 15 min at 100°C in a drying over, weighed and expressed on non-dried feather weight basis.

An indication of the accuracy of this method was obtained in a pilot study. Triple samples of 5 equally treated battery hens (White Leghorns) revealed a coefficient of variation within hens of 0.02-0.09. Among hens, the amount of feather lipids averaged 16.2 (*SD* = 0.8) mg per g feathers.

*Feather sampling and analysis: Feather structure*

From one location (about 7 cm caudally from the shoulder), 5 mature feathers were cut from each hen on the last day of both the treatment and post-control periods. All 40 samples were kept separately in air filled plastic bags.

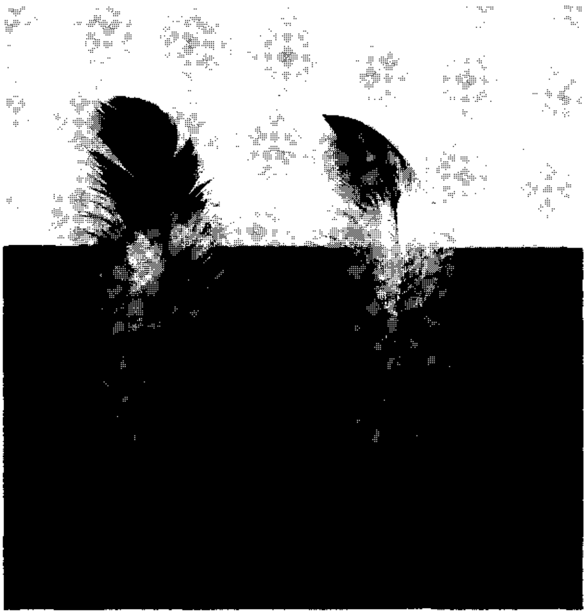

Fig. 1. Back feathers of Warren laying hens classified to be undamaged and fluffy (on the right), and to be damaged and not fluffy (on the left) for structure of pennaceous and downy parts, respectively.

Prior to the actual classification, the origin of the samples had to be masked. Reference feathers were also needed in order to examine feather structure objectively. Thus, all samples were randomly numbered and the origin of each sample was noted. Subsequently, 15 feathers were taken out of randomly chosen bags, and used to determine scoring criteria or to serve as references during classification. Both the distal pennaceous part and the proximal downy part of each feather were examined independently. The pennaceous part was scored to be undamaged when barbs showed a maximum of two indentations, not deeper than half the length of the barbs. The downy part was classified to be fluffy when most of them were standing separately from each other at an angle of 30° or more with the plane of the proximal part of the feather (Fig. 1). A whole sample was judged to be undamaged or fluffy when the bag contained three or more undamaged or fluffy feathers, respectively.

All 40 samples were judged 3 times, with half a day time interval. Hereafter, the origin of the feathers was revealed. Final classification of "undamaged" or "fluffy" was obtained only when whole samples were judged to be undamaged or fluffy at least 2 out of 3 times, respectively. Agreement between sample classifications of pennaceous structure and fluffiness was at the least 95 and 85%, respectively.

### *Behavioural registrations*

During the post-control period, behaviour of the deprived hens was recorded on videotape in order to determine dustbathing frequency and duration. Preceding the present experiment, both the frequency and duration were already assessed for control hens of the second group in the row.

### *Statistical analysis*

Regarding the amount of feather lipids, for control as well as deprived hens. Walsh test statistics were applied on paired samples between all three experimental periods. Both groups were tested against each other, using the Mann-Whitney $U$ test (Siegel 1956).

The binomial test was used in comparing the data of day 36 and day 38 for the number of improvements in pennaceous and downy structure. Between both experimental groups, Fisher exact probability was computed for data on feather structure at day 36 or day 38 (Siegel 1956).

In these statistical analyses, data of one control hen were excluded. This hen had become broody and did not leave the nest box. For control hens sample size was therefore 9, for deprived hens it was 10.

Dustbathing frequency and duration for 5 control hens (pre-experimentally) and 10 deprived hens (during post-control period) were compared with each other with Mann-Whitney $U$ statistics.

## RESULTS

### *Feather lipids*

The amount of lipids on back feathers of control hens did not change significantly over the three experimental periods (Table I). The mean amount of feather lipids remained at about 10 mg per g feathers. The deprived hens started at approximately the same lipid level as the controls. However, during dust deprivation, the amount of feather lipids increased significantly to an average of 14.5 mg per g feathers ($P < 0.01$, Table I). When hens were allowed to dustbathe in the post-control period, the amount of feather lipids declined significantly ($P < 0.01$, Table I) to 10.1 mg per g feathers, which did not differ significantly from the original level in the pre-control period.

Inter-group statistics revealed no significant difference in either pre-control or post-control period. In the treatment period, significantly more lipids were extracted from back feathers of deprived hens than from those of control hens ($P < 0.01$, Table I).

Table I. Mean amount of lipids (with *SD*) in mg per g feathers on back feathers sampled from hens before, at the end of, and after a 33 day dust deprivation period, and from non-deprived control hens. One-tailed Walsh $^{(1)}$, and Mann-Whitney $U$ $^{(2)}$ test probabilities are computed. $^{A\text{-}C}$: $P < 0.01$.

| HENS | SAMPLING PERIOD | | | *N* |
|---|---|---|---|---|
| | PRE-CONTROL | END OF TREATMENT | POST-CONTROL | |
| DEPRIVED | 10.3 (2.0)$^{A(1)}$ | 14.5 (3.0)$^{C(1)(2)}$ | 10.1 (2.7)$^{A(1)}$ | 10 |
| CONTROL | 9.6 (1.6) | 10.9 (2.6)$^{A(2)}$ | 10.4 (2.3) | 9 |

*Feather structure*

Day 38 did not reveal a significant change in pennaceous structure compared to day 36 for either control hens or deprived hens.

Fluffiness of the control group hens did not change significantly between both days either (6 v. 4 fluffy structure scores; 2 hens improved, while 4 deteriorated in feather structure). On the other hand, fluffiness of the deprived hens improved significantly. On the last day of dust deprivation, day 36, only 4 out of 10 deprived hens had a fluffy feather structure. After hens were allowed to dustbathe for 2 days, all 10 ($P < 0.05$, one-tailed) had fluffy back feather down, corresponding to the illustration on the right in Fig. 1.

On day 38, significantly more hens in the deprived group met the criteria regarding fluffiness compared to the control group (10 v. 4 hens; $P = 0.01$, one-tailed). No other significant result was revealed when comparing feather structure scores between groups.

*Behavioural registrations*

Deprived hens dustbathed on average 2.7 ($SD = 0.9$) times during the 2 day post-control period, whereas control hens dustbathed 1.1 ($SD = 0.6$) times in 2 days. This difference is significant ($P = 0.01$, one-tailed). Duration of dustbathing for the deprived hens averaged 29 (*SD* = 4) min, which differed significantly from the control hen dustbathing duration of 20 (*SD* = 8) min ($P < 0.05$, one-tailed).

During the post-control period, control hens and deprived hens only dustbathed in the dust tray and were never seen to perform sham dustbathing behaviour (Wiepkema 1983) on the wooden slat floor. In contrast, deprived hens had performed sham dustbathing during the treatment period, as occasional observations indicated.

## DISCUSSION

In laying hens, dustbathing appears to be essential in the regulation of the amount of feather lipids, since the excess of feather lipids accumulated during dust deprivation is removed shortly after hens are again given access to a dust tray. Supporting evidence concerning the functional significance of dustbathing, suggested by Borchelt & Duncan (1974), is given by the results from the post-control period. Data of the control hens showed that differences in lipid level were not influenced by experimental manipulations such as feather sampling, or uncontrolled factors such as environmental ones.

The mechanism by which feather lipid surplus is removed has not yet been established. It is thought that feather lipids are adsorbed by dust particles tossed between the feathers (Healy & Thomas 1973; Borchelt & Duncan 1974). Dust, with lipids probably attached to it, is removed when the hen terminates a dustbath with a feather shake. Further study of the amount of lipids attached to dust sampled at different phases of dustbathing (Borchelt 1975; Klinger 1985) could indicate how the behavioural elements of dustbathing are functionally related.

In contrast to Healy & Thomas' findings (1973), the present data do not reveal a significant change in the structure of the distal parts of the feathers as a result of dustbathing after dust deprivation. In general, preening contributes to plumage condition (for a review see van Rhijn 1977). Especially regarding the pennaceous structure of the top of a feather, it is very likely that preening interacts with dustbathing, because lipids adhering to he tops of feathers originate from the preen gland (Ishida et al. 1973). If optimal feather top structure can only be achieved by the presence of fresh lipids, then it seems unfortunate that beak-trimmed hens were used in the present experiment, because a clear dustbathing effect on pennaceous structure of the feather top might be obscured by inadequate preening.

Fluffiness of the downy parts significantly increased after a renewed opportunity to dustbathe. Since feather lipids accumulate during dust deprivation, this may imply that a surplus of lipids causes downy barbs to stick together. With the removal of excess of feather lipids, it is possible that down structure is restored after dustbathing. Healy & Thomas (1973) had the same impression, and suggested that the mechanical action of sand being shaken out of the plumage also adds to fluffiness. In contrast, not all control hens showed down parts classified as fluffy, although they were continuously allowed to dustbathe. However, the time interval between dustbathing and feather sampling is very likely to be larger for the control hens compared to the deprived hens, because the deprived hens dustbathed at a higher rate after dust deprivation. This suggests that the downy parts of back feathers only maintain fluffiness for a short period, and deteriorate in structure between two successive dustbaths. Further investigations are required to reveal time dependent changes in fluffiness after a dustbath.

The present results show that dustbathing in the laying hen is functionally significant regarding feather maintenance. If poor feather condition negatively influences animal welfare, then the lack of opportunity to bathe in a dusty substrate, as occurs in battery cages, should be re-evaluated.

## ACKNOWLEDGEMENTS

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## Chapter 2

# DUSTBATHING BEHAVIOUR OF LAYING HENS AS RELATED TO QUALITY OF DUSTBATHING MATERIAL

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# DUSTBATHING BEHAVIOUR OF LAYING HENS AS RELATED TO QUALITY OF DUSTBATHING MATERIAL

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## ABSTRACT

The dustbathing behaviour of laying hens (*Gallus gallus domesticus*) is significantly influenced when either sand or wood-shavings are offered as a dustbathing material. Hens, that are familiar with both materials, prefer sand over wood-shavings as a dustbathing material. This preference is also indicated by the findings that hens increased their dustbathing behaviour when they had been transferred from wood-shavings to sand, whereas a reverse treatment led to a temporary inhibition of dustbathing. For both substrates, short dustbaths (with a modal duration up to 5 minutes) and long dustbaths (of 20-30 minutes) were found. The former corresponded to the introductory (tossing) phase of the latter (complete) dustbaths. The distributions of dustbath durations and the observations of the dustbathing sequences suggest that wood-shavings are less effective than is sand. The efficiency with which feather lipids are removed when hens dustbathe either in sand or in wood-shavings after a 17 day dust deprivation, supports this suggestion.

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## INTRODUCTION

Dustbathing by birds is considered to play a role in the maintenance of the integument (Simmons 1964). As suggested for quail (Healy & Thomas 1973; Borchelt & Duncan 1974) and for hens (Chapter 1), dustbathing removes excessive and/or deteriorated feather lipids, which may improve feather structure. Fresh feather lipids, that originate from the skin or the uropygial gland (Ishida et al. 1973) are thought to keep the feathers supple and water repellent (Elder 1954). However, these lipids can oxidize (Simmons 1964) and accumulate (Borchelt & Duncan 1974; Chapter 1). In a dustbath, the dust particles that have been tossed and rubbed between the feathers are thought to adsorb feather lipids. Feather structure seems to be restored due to both a reduction of sticky lipids and the mechanical combing-like action of shaking when the particles are removed from the plumage (Healy & Thomas 1973).

Little is known about the quality of the material that is required for dustbathing in gallinaceous birds. Hein (1970) indicated that the ruffed grouse prefers sandy dustbathing sites with particles of a specific range in size. Thus, these birds are able to differentiate between sandy substrates, which implies that the choice of a dustbathing substrate depends on specific requirements. Free ranging hens are seen to dustbathe mostly in sand (Fölsch & Vestergaard 1981), whereas group housed hens usually have access to wood-shavings. However, the physical properties of these materials differ considerably. Sand particles are small, though heavy and smooth, and wood-shavings are coarse, light and likely to adhere. This suggests that both substrates do not reach between the feathers in the same way during dustbathing and therefore could differ in their effect on the integument. If hens are able to differentiate among substrates, as the ruffed grouse does, then it is expected that a difference in quality, would be reflected in a clear preference.

Although hens dustbathe both in sand (Fölsch & Vestergaard 1981; Fölsch et al. 1986; Chapter 1) or in wood-shavings (Wennrich & Strauss 1977; Bessei & Klinger 1982), it is not known whether the dustbathing behaviour differs depending on the substrate. As such, hens seem to be able to change their dustbathing behaviour, since periods of dust deprivation increase both frequency and duration of the dustbathing bouts and frequency of the components within a bout (Borchelt 1975; Vestergaard 1982; Chapter 1). However, it is not certain whether hens that are experienced in dustbathing in both sand and wood-shavings, organize their dustbaths differently in the different substrates. Moreover, if the patterning of the dustbath differs in these materials, then it is questionable whether dustbathing behaviour plays the same functional role. As dustbathing removes excessive lipids (Chapter 1), functional comparison between dustbaths in sand or in wood-shavings can be done on the efficiency with which hens remove feather lipids. A significant removal of feather lipids is expected when hens are given access to dust after a period of deprivation (Chapter 1). Therefore, comparison between dustbathing in sand or wood-shavings and the efficiency with which hens remove lipids, is especially interesting in the days following deprivation of dust.

The aim of this study is to investigate the preference for and the tendency and the patterning of dustbathing behaviour in sand or wood-shavings and the difference in the efficiency with which feather lipids are removed when hens are offered one of these materials after a period of deprivation.

## ANIMALS, MATERIAL AND METHODS

### *Animals and housings*

Twenty four beak-trimmed 18-week-old Warren laying hens reared on deep litter were obtained from a commercial dealer. On their arrival, the hens were housed on wood-shavings (averaging 30 mm$^2$ in size), and a 60cm x 60cm x 12cm (length x width x heigth) tray of river sand, which was refilled twice a week, was placed in the pen. After one month, immediately before the experiments, the hens were wing-tagged for individual identification and randomly divided into four groups of six hens. Each group was housed in a 260cm x 150cm x 240cm pen with wooden slat floors and nestboxes, which did not contain any litter. The pens, placed in a single row, were visually isolated from each other and maintained on a 13/11 light/dark cycle (lights on at 0600 hours). Food from a 7 cm wide feeding trough and water were provided ad libitum. Air temperature averaged 18°C; average relative air humidity was 60%.

### *Experimental design*

The study was divided into two experiments (fig. 1). In the first experiment, hens were shifted from wood-shavings to sand and vice versa. A test of the preference for either sand or wood-shavings when both materials are present, followed in the second experiment.

### *Experiment I*

The first experiment lasted 68 days (fig. 1). This experiment was divided into three phases. During phase 1 (12 days) the birds were made familiar with both sand and wood-shavings by presenting these alternately every third day. No other particulate dustbathing medium was available.

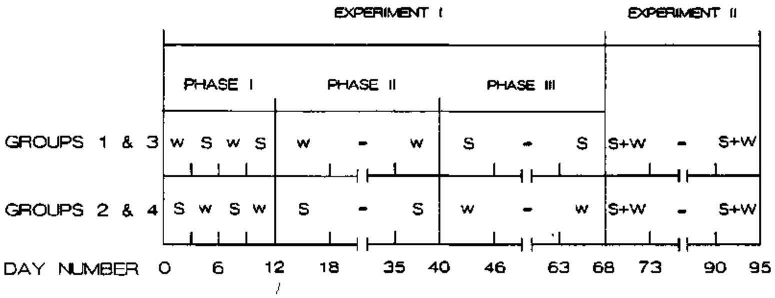

Figure 1. The experimental design of two experiments as performed over 95 days with groups 1 and 3 and groups 2 and 4, respectively. W: the tray in the pen is filled with wood-shavings; S: the tray in the pen is filled with sand; -: no dustbathing material is present, period of deprivation; S+W: half of the tray in the pen is filled with sand, the other half is filled with wood-shavings.

Phases 2 and 3 each consisted of three time periods. During the first 6 day period and the final 5 day period dustbathing substrate was presented; the middle period consisted of a 17 day litter deprivation (fig. 1).

In phase 2, the substrate was wood-shavings for the hens, that had been on sand previously (groups 1 and 3) or sand when they had been on wood-shavings (groups 2 and 4). At the beginning of the deprivation period, the litter was removed from all pens. In phase 3, the conditions were reversed; groups 1 and 3 were offered sand and groups 2 and 4 were offered wood-shavings as a dustbathing material. Although substrate conditions were reversed for groups 1 and 3 and groups 2 and 4, each substrate treatment was thought to be comparable for all 4 groups, since for all groups each treatment was preceded by at least 3 days, in which the other substrate was presented.

### *Experiment II*

The second experiment immediately followed the first and lasted 27 days (fig. 1). Now, both dustbathing materials were presented at the same time during 5 days preceding and 5 days following a deprivation period of 17 days. Both substrates were provided in the original tray, as described earlier, but now the tray was partitioned into two equal compartments with a slat of 35 cm height. One part was filled with sand and the other with wood-shavings. No other litter was provided in the pen. At the start of the deprivation period the tray was removed from all pens.

### *Behavioural observations*

#### *Experiment I*

During the pre- and post-deprivation periods of phase 2 and 3 in the first experiment, the behaviour of all hens was recorded on video from 0830 until 1830 hours and frequencies and durations of dustbaths were noted. Since 90 % of the dustbathing behaviour occurs between 1100 and 1700 hours (Vestergaard 1982), the observation period was assumed to give reliable estimates of daily frequencies and durations of dustbathing.

The dustbathing behaviour as described by Kruijt (1964) and Borchelt (1975) was considered to begin when a hen squatted down and performed vertical wing shaking. The end of a dustbath was determined by the start of an interval of 15 minutes or more which did not include dustbathing behaviour. Sequences that included a shorter interval without dustbathing were considered to belong to one uninterrupted dustbath. This occurred in 9% of the total number of dustbaths.

#### *Experiment II*

In the second experiment, the hens were observed at 15 minute intervals between 1000 and 1600 hours. Number of observations in which hens dustbathed either in sand or wood-shavings was counted for each group and day.

### *Feather sampling and analysis*

In the first experiment samples of feathers were taken on the last day of all three time periods in both substrate treatments. From each hen, ten, apparently clean and mature feathers were cut off at their base from eight fixed locations on the back, breast and thighs. Thus, about 80 feathers per sample (weighing 2-3 g) and 3 x 2 samples per hen were obtained for lipid extraction. Feather lipids were extracted according to the Soxhlett cold extraction method described by the International Standard Organisation (Anonymous 1983). The lipids were extracted from the feathers with diethyl ether for two hours. Hereafter, the solvent was removed by distillation. The lipid residue was dried for 15 minutes at 100°C in a drying oven and weighed after cooling down to room temperature. The amount of feather lipids was expressed per gram (non-dried) feathers. An estimate of the reliability of this method has been given in Chapter 1.

*Descriptive statistics*

Since hens tend to dustbathe in groups (Wood-Gush 1971), and amount of feather lipids is related to amount of dustbathing behaviour (Chapter 1), the data are assumed to be dependent within groups. Therefore, for all estimates, sample size is 4, based on 3 groups of 6 hens and 1 group of 5 hens. In the latter group, the data of one hen were excluded, because this hen's leg was injured.

The following descriptive statistics were carried out for each time period and substrate treatment separately.

*Experiment I*

For each hen, the duration of a dustbath was based on the average of all dustbaths this hen had performed. Thereafter, these estimates were averaged over all hens within a group. In table I, final estimate of dustbath duration is given as an average over the 4 groups.

Daily frequency of dustbathing (table I) was calculated by counting the number of dustbaths performed on each day for each hen. A day count was 0 when a hen did not perform any dustbathing that day. Over the days these sums were averaged for each hen. Then, these estimates were averaged over all hens within each group and thereafter over the 4 groups.

To illustrate the response of the groups after transfer from one litter to the other or after deprivation, mean total time spent dustbathing per day (fig. 2) was calculated. Time spent dustbathing was summed per day for each hen and averaged per day over the hens within a group. Thereafter, these estimates were averaged over the groups and plotted against day number. For each day, total number of hens, that performed one or more dustbaths, is included in the graph.

The frequency distributions of the durations of the dustbaths in sand or in wood-shavings, as well as before and after the deprivation period is presented in fig. 3. These dustbaths have been pooled over all individuals and days, that are involved.

The amounts of feather lipids were averaged over the hens within a group. Extreme values in the amount of feather lipids were identified according to the Nair criterion (Natrella 1966) with 2.0 as an estimate of the standard deviation derived from concurrent data. From a total of 2 x 3 x 23 samples, five values were excluded; two from the 23 samples at the period after deprivation of sand; two from the samples at the period before deprivation of wood-shavings; and one at the period after deprivation of wood-shavings. In table III, amount of feather lipids averaged over the 4 groups is presented.

*Experiment II*

In the second experiment the number of observations which included one or more hens that were dustbathing in either sand or wood-shavings was summed per day and group. Hereafter, these totals were averaged over the days and groups (table II).

### *Statistical analysis*

For both experiments and each behavioural variable, pair-wise comparisons of group estimates were done with a Wilcoxon matched pair signed rank test (Conover 1980). The probability of the test statistic under the $H_0$ was Chi square approximated.

Analysis on the group averages of the amount of feather lipids was performed with a paired *t*-test (Sachs 1984).

Tests were one-tailed for comparisons within substrate treatments between time periods, since it was already indicated what effects on the tendency of dustbathing behaviour and the amount of feather lipids could be expected during and after a deprivation of litter (Chapter 1). Between substrate treatments comparisons were two-tailed.

## RESULTS

### *Behavioural observations*

#### *Experiment I*

All 23 hens were observed to dustbathe on sand both before and after the deprivation. On wood-shavings, 17 and 22 hens were observed to dustbathe before and after deprivation, respectively; time per dustbath was on average significantly longer after deprivation than before deprivation (table I). For hens on sand, this difference was not significant. During the pre-deprivation period, dustbaths on wood-shavings were shorter than those on sand (table I), whereas after deprivation the opposite difference was found (table I).

Table I. Mean duration (min.) of a dustbath and mean daily frequency of dustbaths per hen (with *SD*) for three groups of 6 and one group of 5 Warren laying hens over 6 days before or 5 days after a 17-day deprivation of sand or wood-shavings.

| BATHING MATERIAL | DUSTBATH DURATION | | DAILY DUSTBATH FREQUENCY | |
|---|---|---|---|---|
| | BEFORE | AFTER | BEFORE | AFTER |
| SAND | 20.8 (4.7)$^A$ | 22.9 (1.9)$^B$ | 0.8 (0.3)$^E$ | 1.1 (0.3)$^F$ |
| WOOD-SHAVINGS | 14.0 (4.9)$^C$ | 28.1 (5.3)$^D$ | 0.8 (0.5) | 1.1 (0.4) |

Wilcoxon matched pair probabilities are one-tailed within and two-tailed between dustbathing materials. ($N=4$; $^{A\text{-}C}$, $^{B\text{-}D}$, $^{C\text{-}D}$ and $^{E\text{-}F}$: $P< 0.05$; other comparisons: *NS*).

Following deprivation, daily dustbath frequency increased significantly for hens on sand, but not for hens on wood-shavings (table I). Within time periods and between substrate treatments dustbathing frequency did not differ significantly.

Mean total time spent dustbathing per day before and after deprivation is shown in fig. 2. After the first day of the pre-deprivation period, time spent dustbathing in sand dropped to a relatively constant level. For hens which had been transferred from sand to wood-shavings, the daily tendency to dustbathe increased significantly over the days in the pre-deprivation period (fig. 2; Spearman rank correlation between group totals and day numbers: $r_S = 0.63$, $P < 0.001$). On the first day of the pre-deprivation period, the difference in total time spent dustbathing per day differed significantly between the sand and the wood-shaving treatments (fig. 2, $P < 0.05$), but did not on the sixth day.

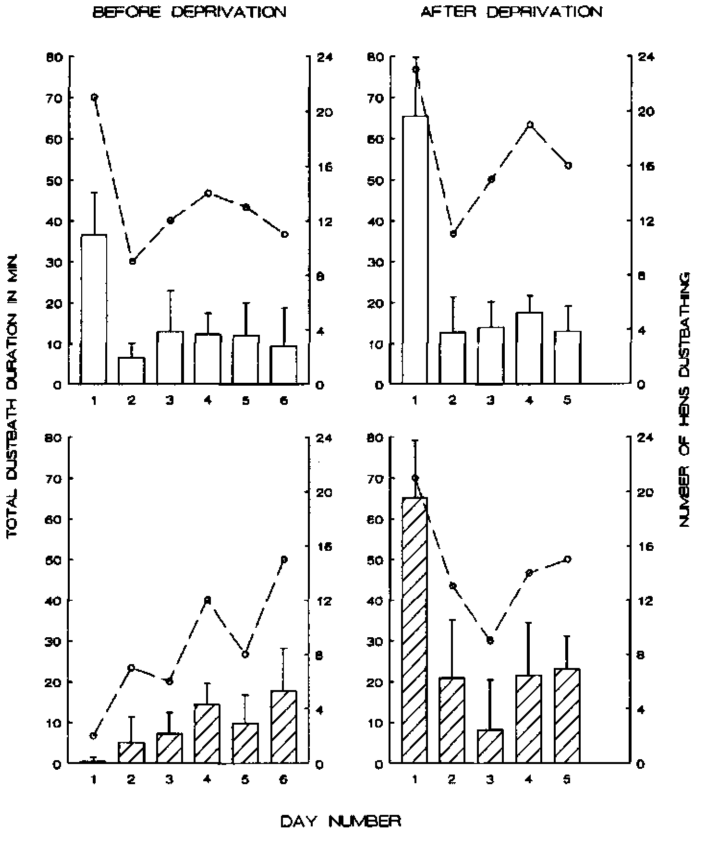

Figure 2. Mean total time spent dustbathing per day (in bars with *SD*) and total number of hens dustbathing (dashed line) over (left graphs): 6 days after transfer from wood-shavings or sand, respectively, and just before the 17 day deprivation period; (right graphs): 5 days immediately after the deprivation period. Open bars: dustbaths in sand; hatched bars: dustbaths in wood-shavings. Further explanation is given in the text.

Immediately after deprivation hens spent significantly more time dustbathing compared to the last day before deprivation (fig. 2, $P < 0.05$ for both sand and wood-shavings). After the first day of the post-deprivation period the daily tendency to dustbathe dropped to a relatively constant level in both treatments. On the first and the fifth day after deprivation total time spent dustbathing did not differ significantly between the substrates. Similar results were found for the number of hens that performed dustbathing behaviour in the subsequent days of the pre- or post deprivation periods (fig. 2).

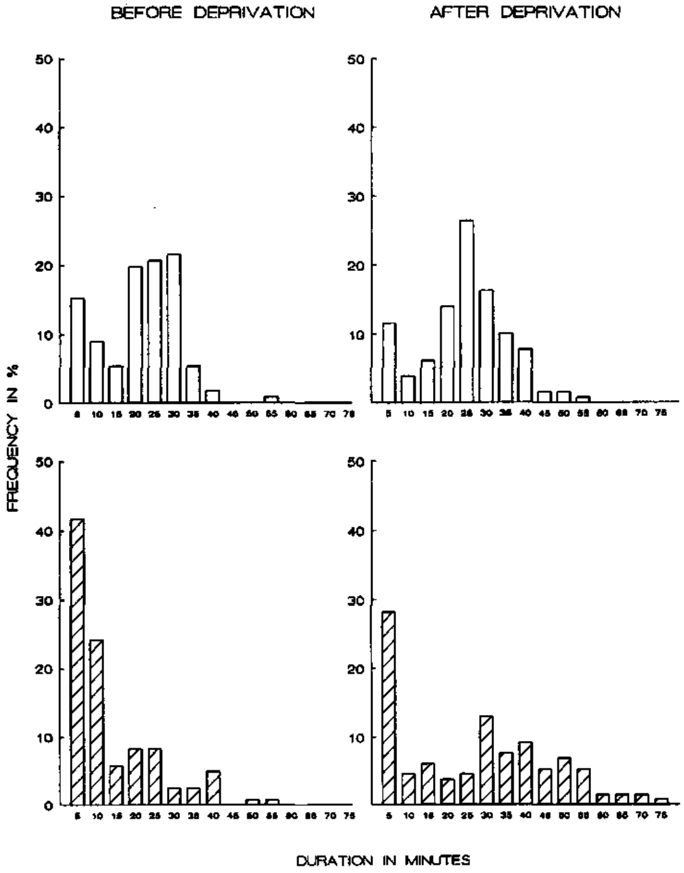

Figure 3. Distribution in 5-min. classes of dustbath durations of hens on sand (open bars) or on wood-shavings (hatched bars), performed before (left graphs) and after (right graphs) a 17 day deprivation of this dustbathing material. $\Sigma n_i = 111$ ($i=1,..23$), or $\Sigma n_i = 120$ ($i=1,..17$) before deprivation and $\Sigma n_i = 129$ ($i=1,..23$), or $\Sigma n_i = 131$ ($i=1,..22$) after deprivation in sand and wood-shavings, respectively, for dustbaths of a maximum of 23 hens.

As illustrated in fig. 3, dustbathing behaviour seems to be heterogenous with regard to duration. The bimodal distribution found for hens on sand in the pre- and post deprivation period and hens on wood-shavings in the post-deprivation period indicates the occurrence of both short dustbaths with a modal duration of 0 to 5 minutes and longer dustbaths with a modal duration of 20 to 30 minutes. Although distributions as given here may be biased due to differences between individuals, short and long dustbaths were observed for each individual. However, in the pre-deprivation period, hens on wood-shavings mostly have short dustbaths, which results in an exponential-like distribution.

The short dustbaths in sand and wood-shavings did not differ strikingly in terms of the behaviour performed. The introductory behaviour consisted of scratching with two legs, while squatting and bill raking. A tossing sequence followed, in which head rubbing, scratching, vertical wing shaking and bill raking was performed with fluffed feathers, while lying on the breast or side. The termination of the short dustbaths occurred at irregular intervals when the hen stood up and initiated other behaviour.

The long dustbaths in sand or wood-shavings differed from the short ones in the inclusion of lying on the side with flattened feathers and side rubbing behaviour. These behavioural elements always occurred after several bouts of tossing behaviour, which were performed in a way similar as just described for short dustbaths. Standing up again and shaking the feathers always marked the end of the long dustbath. Within the long dustbaths a difference was observed between the patterning of baths in sand and of baths in wood-shavings. In sand, long periods (up to 10 to 15 minutes) of side rubbing and lying on the side were performed, which never occurred on wood-shavings. In the long dustbaths in wood-shavings, bouts of lying on the side and side rubbing were much shorter (up to 3 minutes) and were frequently interrupted by reinitiated tossing behaviour. This alternation between rubbing and tossing sequences also accounted for the extremely long dustbaths in wood-shavings (40 minutes or more).

The effect of the tossing behaviour was observed to differ between both materials. Sand was easily tossed among the feathers and reached the skin of the back, belly and wings. Wood-shavings were tossed between the proximate parts of the feathers and were only seen to reach the skin in the featherless spaces (e.g. the uropygial eminence and the adjacent area; Lucas & Stettenheim 1972a).

### *Experiment II*

During both the 5 days before and the 5 days after deprivation, hens showed a clear preference for dustbathing in the sand compartment of the tray. Average number of times per day hens were observed to dustbathe in sand differed significantly both before and after deprivation from the number of times per day hens dustbathed in wood-shavings (table II). The number of times per day hens were observed to dustbathe after deprivation was significantly higher than before deprivation for dustbaths in sand (table II), but not for dustbaths in wood-shavings.

Table II. Mean number of observations (with *SD*) in which hens dustbathed in either sand or wood-shavings when presented with both substrates, for three groups of 6 and one group of 5 Warren laying hens, in 24 observations per day over 5 days before and 5 days after a 17 day deprivation of both dustbathing materials.

| BATHING MATERIAL | NUMBER OF OBSERVATIONS | |
|---|---|---|
| | BEFORE | AFTER |
| SAND | 8.8 (1.9)$^{A}$ | 10.6 (2.3)$^{B}$ |
| WOOD-SHAVINGS | 0.2 (0.2)$^{C}$ | 1.0 (0.7)$^{D}$ |

Wilcoxon matched pair probabilities are one-tailed within and two-tailed between dustbathing materials. ($N=4$; $^{A\text{-}B}$, $^{A\text{-}C}$ and $^{B\text{-}D}$: $P < 0.05$; $^{C\text{-}D}$: *NS*).

## *Feather lipid analysis*

The amount of feather lipids did not differ significantly between substrate treatments in any of the three sampling periods. Both hens deprived of sand and hens deprived of wood-shavings showed a significant accumulation of feather lipids at the end of the deprivation period (table III). However, after renewed opportunity to dustbathe for 5 days, the amount of feather lipids was significantly lower (table III) and this did not differ from the original level just before dust deprivation.

Table III. Mean amount of feather lipids (mg lipids $g^{-1}$ feathers; with *SD*) of four groups of Warren hens' plumage (23 birds in total) sampled at the last day before, at the end of and 5 days after a 17-day deprivation period of sand or wood-shavings.

| BATHING MATERIAL | AMOUNT OF FEATHER LIPIDS | | |
|---|---|---|---|
| | BEFORE | AT THE END OF | AFTER |
| SAND | 7.7 (1.0)$^{A}$ | 9.0 (0.9)$^{B}$ | 8.0 (1.9)$^{A}$ |
| WOOD-SHAVINGS | 8.0 (0.6)$^{A}$ | 8.9 (0.8)$^{B}$ | 8.3 (0.9)$^{A}$ |

Paired *t*-test probabilities are one-tailed within and two-tailed between dustbathing materials. ($N=4$; $^{A\text{-}B}$: $P < 0.05$).

## DISCUSSION

On the first day of the pre-deprivation period, when hens have had wood-shavings as a dustbathing medium, the tendency to dustbathe in sand is much higher than in the subsequent days (fig. 2). This contrasts sharply with the response on the first day after hens had been transferred from sand to wood-shavings. In the latter case, hens hardly dustbathed (fig. 2). Apparently, hens used to sand are reluctant to dustbathe in wood-shavings when this substrate is given after sand, and show a rebound-like effect when put back from wood-shavings on sand. This suggests that wood-shavings are less attractive as a dustbathing material than is sand. The second experiment confirms this suggestion. It showed that hens differentiate between substrates and consistently choose sand over wood-shavings when both substrates are presented at the same time (table II).

After the first day of transfer, hens on sand dustbathed at a steady level, whereas on wood-shavings, the daily tendency to dustbath increased with time (fig. 2). Thus, early aversion may have diminished or may have been overruled by stimulating factors that increase in strength over the days in the pre-deprivation period. The latter suggestion corresponds with Vestergaard's finding (1982) that the tendency to dustbathe after deprivation of dust increases with daily lengthening of the preceding deprivation period. Since most hens were not observed to dustbathe immediately after transfer from sand to wood-shavings (fig. 2), the increase in the tendency to dustbathe may be comparable to that which occurs during a real deprivation of dust.
Despite the clear cut preference observed in the second experiment, the first experiment showed, that in the pre-deprivation period the daily frequency of dustbathing did not differ between the sand and the wood-shavings (table I). However, a distinction in the tendency to dustbathe in sand or wood-shavings, is suggested by the difference in dustbath organisation on sand and wood-shavings.

The bimodality in dustbath duration found for dustbaths in sand or wood-shavings after deprivation (fig. 3) suggested the existence of short and long dustbaths. A short dustbath, which only includes tossing behaviour, could serve a particular function or could be comparable to the introductory phase of a long dustbath. In the latter view, the tossing behaviour sets the conditions for subsequent rubbing behaviour. This is plausible because rubbing is always preceded by tossing behaviour (this study; Kruijt 1964; Borchelt 1975; Fölsch et al. 1986). In a functional context this may be relevant, since the feathers are flattened and the wings are held tightly to the body while rubbing (this study, Kruijt 1964; Borchelt 1975), which enables a hen to enclose the particles that previously have been tossed between the feathers and to enhance the contact between the particles and the feathers or skin. However, the effect of tossing behaviour differs between dustbaths in sand and wood-shavings. As hens dustbathe in wood-shavings, particles hardly reach between the feathers, whereas in sand, the particles reach the skin. If rubbing behaviour is causally dependent on the effect of tossing behaviour, then the tendency to perform rubbing behaviour may differ between the wood-shavings and sand treatment. This suggestion is supported by our finding that hens mostly perform short dustbaths when given wood-shavings, whereas on sand they perform more long dustbaths (fig. 3). A preliminary conclusion could be that rubbing behaviour is inhibited in wood-shavings, although further research on causal and functional relationships between the short and long dustbaths is needed to verify this. Moreover, more detail is needed to test what physical properties of the substrate are involved in the patterning of dustbathing.

On the first post-deprivation day, hens on either sand or wood-shavings showed a much higher tendency to dustbathe than before deprivation (fig. 2). This is a well known phenomenon (Wennrich & Strauss 1977; Vestergaard 1982; Chapter 1), which is also apparent in the overall estimates of the duration of a dustbath or the daily frequency of dustbathing (table I). Although the tendency to dustbathe is higher after deprivation than before deprivation, the preference for sand over wood-shavings remained clear cut (table II). However, despite the conclusion that wood-shavings represent a less attractive bedding than sand in which to dustbathe, hens in wood-shavings took longer dustbaths than hens on sand, after deprivation (table I). Paradoxically, it could be concluded that after deprivation hens show a higher tendency to dustbathe on the less preferred wood-shavings. However, after deprivation on wood-shavings, hens seem to take more short and extremely long dustbaths than on sand (fig. 3). As such, the latter finding suggests that the dustbathing response in wood-shavings is ambivalent, which could correspond with the organization of the long dustbaths in wood-shavings. In contrast to the long dustbaths in sand, tossing and rubbing behaviour in the wood-shavings seem to alternate, as short bouts of rubbing behaviour are more frequently interrupted by bouts of tossing behaviour. A dithering between these behavioural elements may occur when the tendency to perform tossing and rubbing behaviour is high, although the effectiveness of both the tossing and the rubbing behaviour is too low to sustain a prolonged bout (Houston & Sumida 1985). A longer study on the development of the organization of bathing behaviour in wood-shavings would clarify whether this dithering continues or develops in a more consistent bathing behaviour. If dustbathing in wood-shavings is less effective, which causes the tendency to dustbathe to decrease slowly, compared to dustbathing in sand, then the afore mentioned paradox may simply be explained by the idea that hens on wood-shavings need more time to reach the same effect. Indeed, in the 5 day period following deprivation, hens spent more time bathing in wood-shavings than in sand (table I), whereas the removal of featherlipids was virtually the same (table III). These findings fit with the assumption that dustbathing maintains feather lipid homeostasis (Levine et al. 1974), although it is still to be solved whether feather lipid content and amount of dustbathing in wood-shavings or sand remain at the levels as found in this study over a long period of time. Moreover, the causal effects of quality (Simmons 1964) and/or quantity of feather lipids (Chapter 1) on dustbathing behaviour are not clear, which will be investigated in a later experiment.

Despite the presumed difference in the efficiency with which feather lipids are removed, ultimate amount of lipids, that was still present on the feathers, seemed to be comparable between both materials (table III). Therefore, it could be argued that dustbathing in either sand or wood-shavings does not differ functionally with regard to feather lipid homeostasis. However, it is not known if the distribution of feather lipids over the single feathers is affected in the same way, since sand particles seem to penetrate more between the feathers than wood-shavings do. A more detailed study will be needed to clarify this.

## ACKNOWLEDGMENTS

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## Chapter 3

# OILING BEHAVIOUR AND THE EFFECT OF LIPIDS ON DUSTBATHING BEHAVIOUR IN LAYING HENS

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# OILING BEHAVIOUR AND THE EFFECT OF LIPIDS ON DUSTBATHING BEHAVIOUR IN LAYING HENS

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## ABSTRACT

Laying hens oiled the plumage twice a day, while oiling behaviour consisted of a bout of 5 oilings (median value). During one oiling a hen collected lipids from the preen gland with her bill and subsequently performed (as a median) 5 strokes or rubs over and through the feathers. The breast was oiled most, whereas during later oilings within an oiling bout the wings and the flanks were oiled as well. The back and tail were seldom oiled. An artificial distribution of stale uropygial gland lipids in a more or less natural way on the breast feathers within a 3 day period of sand deprivation resulted in an increase of the duration of the first dustbath after the deprivation by 12 %, compared to a control treatment. Within the dustbath, the total number of the side lying and side rubbing elements -two related consummatory dustbathing elements- doubled and tripled, respectively. In contrast to these highly significant effects, the duration of the dustbathing did not change after a fresh uropygial gland lipid treatment, while the effects on side rubbing and side lying were not straight forward; the total number of the side rubbing element was doubled, but the duration of side lying was reduced by 29 %. The results are discussed in relation to the chosen methodology and the maintenance of the lipid condition of the integument.

## INTRODUCTION

Lipids on the feathers of birds serve essential functions such as adequate water proofing and reduction of wear or chances of feather breakage (Elder 1954). During oiling, uropygial gland lipids are dressed onto the feathers (Simmons 1964; McKinney 1965; van Rhijn 1977), but feathers also receive chemically similar lipids as a product of skin keratinization (Bolliger & Gross 1958; Bolliger & Varga 1960; Lucas & Stettenheim 1972b; Ishida, et al. 1973; Borchelt et al. 1979). The amount of feather lipids may become excessive, for instance in quail after a period of five days without dustbathing litter (Borchelt & Duncan 1974). In hens, excessive lipids on the plumage were removed within two days following a 33 day period of sand deprivation (Chapter 1). As hens dustbathe longer and more frequently following deprivation compared to the preceding level, it has been suggested, that a high content of lipids on the feathers could stimulate dustbathing behaviour (Borchelt et al. 1973; Levine et al. 1974; Vestergaard 1982; Chapter 1 and 2). In 1979, Borchelt et al. tested this hypothesis. They increased artificially the amount of lipids on the plumage of quail, but did not find a clear effect on dustbathing behaviour thereafter. However, dustbathing behaviour is not only affected by peripheral factors (Vestergaard et al. 1990), and the amount of lipids on the feathers per se need not be the only concerning feather lipid condition; the following considerations could be relevant. Firstly, Borchelt et al. sprayed a chloroform-methanol solvent with lipids over the plumage, producing an even distribution of the lipids over the surface of the plumage after evaporation of the solvent. However, such a distribution may not correspond with the one following normal oiling behaviour. Uropygial gland lipids have been described to be distributed in lumps over the distal parts of pennae and plumuli in roosters that had been beak-trimmed (Ishida et al. 1973). Further, the distribution of lipids over the different parts of the plumage may strongly differ. If some parts of the plumage are oiled more or differently than others, as is the case in gulls (van Rhijn 1977) and several species of Anatidae (McKinney 1965), then a spray of lipids over the whole bird may lack normal consequences. Therefore, it may be useful to base the methodology of applying lipids onto the plumage on the way birds (that dustbathe regularly) perform oiling behaviour. As no detailed reports about oiling behaviour of hens are known, we shall first describe oiling behaviour in laying hens.

Secondly, Borchelt et al. (1979) used feather and uropygial gland lipids of unknown quality. However, the quality of the lipids that accumulate on the plumage during dust deprivation, is likely to change over time, as lipids on the feathers become stale (Simmons 1964). Therefore, the quality of the lipids on the feathers may also causally relate to dustbathing behaviour after dust deprivation.

In this study, we recorded normal oiling behaviour in hens that dustbathed regularly and simulated some features of hen's oiling behaviour when applying lipids artificially onto the feathers. Either fresh or stale lipids were applied during a short-term deprivation in order to test their respective effect on subsequent dustbathing behaviour.

## OBSERVATIONS OF OILING BEHAVIOUR

### *Animals, Material and Methods*

Oiling and dustbathing behaviour of 10 Warren laying hens, housed in four groups of either two or three hens, were observed for five consecutive days in the 21st or 25th week of age by means of whole-day video recordings. Until 18 weeks of age these hens had been reared commercially in battery cages (the beaks were kept intact). Each group was housed in a 200cm x 170cm x 180cm (l x w x h) pen with a wooden slat floor, which included a nest box, provided with wood-shavings and a 60cm x 60cm x 12cm dust tray with river sand. This tray was refilled twice a week. The pens were placed in a single row, and the groups were visually isolated from each other. Food and water were provided ad lib. and a 15-9 hour light-dark cycle was maintained (lights on at 0500 hours). Temperature averaged 15°C; average humidity was 50%. Due to large variation and unknown distribution of the different dependent variables, the median and range, or in case of a graph, third quartile deviation (which is the median-third quartile range) were chosen as descriptive parameters for the samples based on all (10) individual estimates. Unless explained otherwise, each individual estimate was the median over all observations within the individual.

*Results*

Oiling behaviour occurred mostly once in the three hours after onset and once before offset of the light (31 % and 36 % of all oiling behaviour, respectively; cf. Wiepkema 1989). Median daily frequency per hen was 2 (range: 1-3). Oiling behaviour was performed in a bout of 5 successive oilings (median value; range: 3-6). Each oiling comprised one gland manipulation followed by a variable amount of strokes and rubs over and through the feathers. A representative example of one oiling bout with 4 oilings is shown in fig. 1.

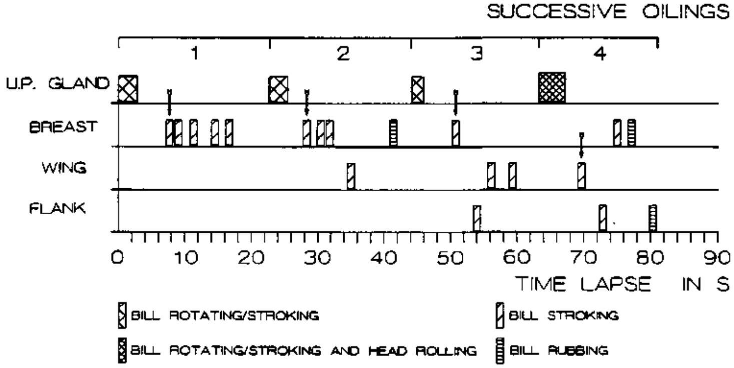

Figure 1. Diagram of a representative sequence of one oiling bout, in which the type and duration of the movements is illustrated for the successive alternations between the uropygial gland and plumage (4 oilings) within that bout. No other contact between bill and gland or plumage was observed. (Arrows indicate what part of the plumage is stroked or rubbed immediately after gland manipulation; see the text for further explanation).

Each oiling started with a characteristic posture, in which the hen extended the bows of her wings laterally and uncovered the nipple of the uropygial gland by erecting the feathers, that were located at the posterior rim of the dorsal caudal tract (Lucas & Stettenheim 1972a). While the hen turned her head laterally to reach the gland from the side, the tail was turned ventro-laterally towards the same side. Subsequently, the uropygial gland was manipulated by rotating and stroking movements of the bill against the nipple of the uropygial gland. During this manipulation, the bill was either closed or slightly opened, and lipids became adhered onto the top of the bill. It was not clear whether the lipids were already present at the top of the nipple or whether manipulating the nipple caused the excretion of the lipid. Towards the end of an oiling bout, the oilings could also include a manipulation of the uropygial gland by a rolling of the head and the anterior part of the neck over the nipple of the gland. It could be that in this way head and anterior neck were dressed with oil. Such head rolling at the end of the oiling bout has also been described in Anatidae (McKinney 1965). After each manipulation of the gland, the hen quickly turned the bill to the plumage.

The movements of the bill immediately following manipulations of the gland were performed in a proximal-distal direction along the feathers and were not restricted to one small locality of the plumage. The tip of the closed bill could be either moved through the feathers (stroking) or the bill could be moved laterally over the feathers (rubbing). A median value of 5 stroking or rubbing movements (range: 4-7) was found during one oiling. During the later oilings of the oiling bout several parts of the plumage may be frequented (fig. 1). Following an oiling bout, hens mostly initiated preening behaviour, which involved bill movements of relatively small amplitude at a small locality of the plumage.

The following parts of the plumage that were oiled, were distinguished on the basis of the topographic anatomy of Lucas & Stettenheim (1972a): the breast (the posterior ventral region up to halfway the proventer region ), the wings (the frontal and the dorsal part), the flanks (both lateral parts of the trunk, that extend between the shoulder backwards to the posterior end of the body and include the ventral surface of the wings), the back (the dorsal part between the neck and the tail) and the tail. To estimate the intensity, with which each hen oiled these different parts of the plumage, we noted per oiling what part was stroked or rubbed first, immediately following a manipulation of the uropygial gland. In fig. 1 these locations are indicated with arrows. These counts were thought to be reliable, as it was always possible to observe a hen moving her bill from the uropygial gland towards some part of the plumage, irrespective of her position towards the video camera. However, subsequent strokes or rubs within one oiling could not be counted reliably when they were performed with a relatively small amplitude and oriented away from the camera. Oilings could be observed completely only when the hen was oriented towards the camera. This was the case for those in the oiling bout of fig. 1.

The number of first strokes or rubs were summed per successive oiling within an oiling bout, per part of the plumage, and per hen for all oiling bouts that had been observed, and expressed in relative frequencies. Since all hens performed at the least one bout with 7 oilings, the probability of stroking or rubbing the different parts of the plumage immediately after manipulation of the uropygial gland was estimated for up to 7 successive oilings. Median probability (and third quartile deviation) was calculated over the hens and plotted for each part of the plumage against the successive oilings within one oiling bout (fig. 2). The results for the back and the tail region are not included in fig. 2, since the median probabilities as well as third quartile deviations for these parts to be stroked or rubbed first were zero for all oilings. In the 89 observed oiling bouts including 458 oilings, only 5 and 2 first strokes or rubs had been directed to the back and tail, respectively. Moreover, the back and tail were seldom treated during subsequent strokes and rubs within one oiling.

Figure 2 shows that over all successive oilings, most first strokes or rubs were addressed to the breast ($P < 0.05$; Friedman test). Furthermore, from the second and the fourth oiling on, the wings and the flank were likely to be treated, respectively. For the wings this probability was highest for the sixth and seventh oiling.

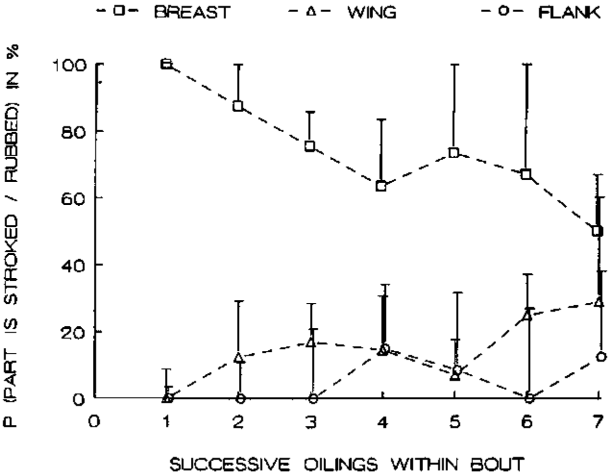

Figure 2. Median probability in % (with third quartile deviation) of stroking or rubbing the bill along the breast, wing, or flank part of the plumage immediately following manipulation of the uropygial gland for the successive oilings within one oiling bout. (N = 10).

During the observations, 80 % of all 66 dustbaths (as defined in Chapter 2) occurred between 1100 and 1700 hours, and all dustbaths were performed in the sand tray. Total number of days in which a hen dustbathed as well as oiled was 3 out of 5 (median value; range: 2-5). Per hen median daily dustbathing frequency was 1 (range: 0-3), whereas median dustbath duration was 16.8 (range: 0.9 - 39.1) minutes. Since both short (less than 5 min.) and long dustbaths (5 min. and longer) exist, while only the long dustbaths include all dustbathing elements (Chapter 2), median daily frequency and median dustbath duration were calculated for the long dustbaths separately. Long dustbaths occurred 0.5 (range: 0-1) times per day per hen with a duration of 23.3 (range: 10.0 - 39.1) minutes.

*Experimental implications*

Firstly, hens oil the breast part of their plumage most. If the location of the feather lipids is crucial in eliciting feather maintenance activities, then the lipid condition of the breast may have a dominant causal effect on dustbathing behaviour as compared to other regions of the plumage. Therefore, we manipulated the level of lipids on the breast feathers only in order to influence dustbathing thereafter.

Secondly, a hen's closed bill with lipids present on its tip, is swiftly moved over and through the feathers, which seemed to treat the feathers in a somewhat irregular way. Thus, we tried to simulate this way of distributing lipids by stroking and rubbing an artificial bill with lipids adhered to its tip along the feathers of the breast.

## THE EFFECT OF LIPIDS ON DUSTBATHING BEHAVIOUR

### *Animals, Material and Methods*

*Animals and housing*

Fifteen White leghorn laying hens with intact beaks were reared and housed in wire cages until the age of seven months. Thereafter, the hens were housed on wood-shavings. At the age of 10 months the hens were randomly divided into three groups of five birds and housed in 150cm x 260cm x 260cm (l x w x h) pens with wooden slatted floors and nest boxes, which did not contain any litter. In the corner of each pen, a 60cm x 60cm x 12cm tray was enclosed within a wire cage of 63cm x 63cm x 50cm, which could be entered through a lockable sliding door. The tray was filled with river sand and refilled every week. The pens were placed in a row, and the groups were visually isolated from each other. A 14/10 light/dark cycle (lights on at 0600 hours) was maintained. Food and water were provided ad lib.. Air temperature averaged 18°C; average relative air humidity was 52%. The experiment started after 10 days of acclimatization. During the latter period, all animals had free access to the tray, and only dustbathed in the sand.

*Experimental design*

The study covered two trials of three weeks. In the first week of the first trial, the hens were locked out of the sand tray for three days (from 1100 hours at day 0 until the start of the observations at day 3). On day 2 at about 0800 hours, all hens in a group were given either a fresh lipid, a stale lipid or a control treatment (see below). One day later (day 3), each hen was permitted to dustbathe one after the other (see section Behavioural observations). After all the hens were given the opportunity to dustbathe in the sand tray, the sliding door of the enclosure was unlocked for the remaining three and a half days of that week. The latter period, in which sand could be used ad lib., was thought to be sufficient to permit the hens to recover from the lipid treatment and deprivation, as hens, that were housed in a comparable way dustbathed 0.6 to 0.8 times a day even without prior dust deprivation (Chapter 1 and 2). In the second and the third week, the procedure was repeated, but per week each group of hens was assigned a treatment, which the hens had not received earlier. In the subsequent trial of three weeks, the whole design was repeated, but now a different order of treatments over the groups and weeks was chosen. This repetition was designed to enhance the reliability of the estimates.

In the case of the lipid treatments, uropygial gland lipids were used. Five months prior to the experiment, these lipids had been collected from six week old broiler pullets by squeezing the uropygial gland base-topwards immediately after decapitation. Subsequently, the lipids were either stored airtight at -20°C (fresh lipids) or kept evenly distributed on a plastic board in a ventilated room at about 20°C for five months (stale lipids). These five months were chosen to ascertain a change in lipid quality, as suggested by Simmons (1964). Indeed, the color of the lipids on the board had changed gradually from butter-like light yellow to grayish yellow. At treatment, three times three strokes in the proximal-distal direction through the breast feathers were performed with a plastic tip (tip width of 3 mm extending to 7 mm at 12 mm length), after dipping just the top into a cup of lipids, which was kept at 37°C. This temperature was chosen arbitrarily between that of the body (41°C) and that of the surface of the uropygial gland (33°C). In total an amount of about 30 mg lipids (three droplets of about 3 mm across) was distributed from the tip onto the feathers. As the breast had about 190 feathers (total weight is 5 to 6 g), 30 mg lipids were estimated to correspond with an accumulation of breast feather lipids, due to about one to two months of dust deprivation (cf. Chapter 1). Despite the irregular distribution, no remains of lipids on the breast feathers could be seen. In the case of the control lipid treatment, no lipids were applied to the tip, but except from that the procedure was the same.

*Behavioural observations*

On day 3 of each experimental week each hen was permitted to dustbathe one after the other. When a hen was in close proximity to the sliding door of the enclosure, the door was opened; after she had entered, the door was closed immediately. In this way each hen was temporarily kept isolated in the sand tray, while visual and auditory contact with hens outside the enclosure was possible. As soon as the sliding door was closed the observation of the hen in the sand tray started. The observation was recorded continuously with an OS-3 microcomputer (Observational Systems Inc., Seattle, Washington). Dustbathing behaviour was defined to begin at first occurrence of vertical wing shaking; the end of a dustbath was marked by body/wing shaking. In a dustbath, vertical wing shaking, bill raking, head rubbing, and scratching with one leg were mostly performed when lying with the plumage fluffed, whereas side lying and side rubbing were performed while the feathers and wings were flattened against the body. Detailed descriptions of dustbathing elements are given by Kruijt (1964); Borchelt (1975) illustrated them (though for Bobwhite quail). Minimum duration which could be measured reliably, was one second. As either bill raking, vertical wing shaking or scratching movements mostly succeeded within one second, each sequence of the same movements was recorded as one bill raking, vertical wing shaking or scratching element, respectively. When a hen had finished her dustbath, the observation stopped, and the enclosure was opened to permit the hen to leave the enclosure. If a hen did not start dustbathing within 15 minutes, the observation was also ended. All observations were done between 1100 and 1700 hours, since most dustbaths (80-90 %) are likely to be performed in this part of the photo period (cf. also Vestergaard 1982).

*Statistical analysis*

For the latency to start dustbathing, duration of the dustbath, and duration and total number of the dustbathing elements, the data of both trials were averaged within treatments and individuals. These averages formed the input for the descriptive statistics and final statistical analysis. As all hens were kept individually during dustbathing, sample size was based on the number of birds. However, the data of one hen were excluded from the statistics, as she never dustbathed on all six occasions she was allowed to dustbathe; therefore sample size was 14. For the fresh and the stale lipid treatment in the first trial and the fresh lipid and the control treatment in the second trial 2, 2, 1 and 2 hens did not dustbathe, respectively. In these cases, latency time was taken to be 15 minutes, while the statistical input of the other dependent variables was based only on the data of the dustbath after the corresponding treatment in the other trial. For the same reason as given in the "Observations of oiling behaviour" part of this study, median and the third quartile deviation, were chosen as descriptive parameters. A Friedman test (Conover 1980) was used to test an overall treatment effect. In case of significance, between treatment differences were analyzed for the three possible pairs of treatments using a Wilcoxon matched pair test (Conover 1980). All tests were two-tailed.

### *Results*

All dustbaths at day 3 belonged to the category "long" and included all dustbathing elements (see the previous "Results" section).

The latency to start dustbathing did not differ significantly between the three treatments (table I), although latency for the hens that were not treated with lipids seemed to be the longest.

**Table I. Median latency and median duration in minutes (with third quartile deviation) of dustbathing behaviour in sand following a three day dust deprivation, that included a control treatment without any lipids, or a treatment with either fresh or stale uropygial gland lipids.**

| | LIPID TREATMENT | | |
|---|---|---|---|
| | CONTROL | FRESH | STALE |
| LATENCY | 2.6 (5.6) | 1.8 (5.4) | 1.7 (3.6) |
| DURATION | 21.6 (5.1)$^{A}$ | 22.8 (4.2)$^{B}$ | 24.3 (7.3)$^{C}$ |

Two-tailed Wilcoxon matched pair probabilities: $^{A\text{-}B}$: *NS*; $^{B\text{-}C}$: $P < 0.05$; $^{A\text{-}C}$: $P < 0.01$; $N = 14$.

The duration of the dustbaths differed significantly amongst the treatments ($P < 0.01$, Friedman test). When the hens were treated with stale lipids, dustbath duration was significantly longer than found after a fresh lipid or a control treatment ($P < 0.05$ and $P < 0.01$, respectively, Wilcoxon matched-pair test). Between the fresh lipid and the control treatment the durations did not differ significantly (table I).

The durations of head rubbing and side rubbing were about one second, of scratching with one leg and vertical wing shaking were about two seconds, whereas the duration of bill raking was about four seconds. No significant differences between the three treatments were found, except for the duration of side lying (fig. 3; $P < 0.05$; Friedman test). Between the control and the fresh lipid treatment the difference was significant ($P < 0.05$; Wilcoxon m.p. test), whereas the stale lipid treatment resulted in an intermediate level that did not differ with those after the other treatments.

The total number of bill rakes as well as scratches was about 80, the total number of vertical wing shakes was about 32, and that of head rubs was about 53. For these elements, total numbers did not differ significantly amongst the three treatments. However, the total number of the side lying and side rubbing elements differed strongly between the treatments (fig. 3; both elements: $P < 0.001$; Friedman test). As the total number of the side lying element doubled and that of the side rubs tripled after stale lipid treatment compared to control treatment (fig. 3; both comparisons: $P < 0.001$), it is thought that these changes have contributed mainly to the increase in the duration of the dustbath. Compared to the fresh lipid treatment the increases were also significant (fig. 3; $P < 0.001$ and $P < 0.01$, respectively). Fresh lipid treatment increased only the total number of side rubs compared to the control treatment (fig. 3; $P < 0.05$; Wilcoxon m.p. test).

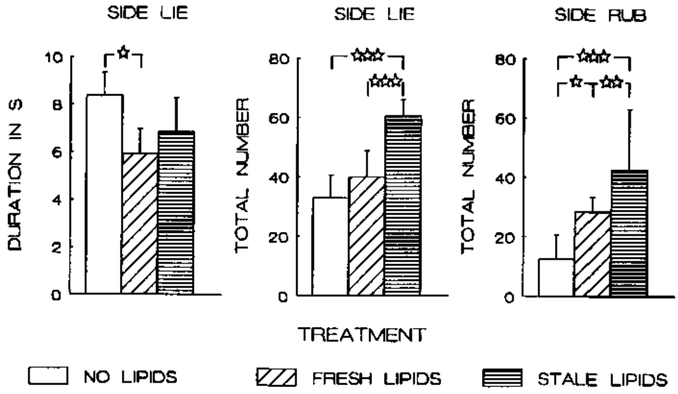

Figure 3. Median duration in seconds and median total number of the side lying element, and median total number of side rubs (with third quartile deviations) in sand, following a three day dust deprivation, that included a treatment without any, with fresh or with stale lipids. Two-tailed Wilcoxon matched pair test probabilities are given (*: $P < 0.05$; ** $P < 0.01$; ***: $P < 0.001$; N = 14).

## GENERAL DISCUSSION

In hens, oiling behaviour occurred in bouts of approximately five oilings and mainly took place twice a day, within three hours after the start and before the end of the photo period. During the oiling bout, lipids were distributed from the uropygial gland onto the plumage in a pattern in which anterior parts preceded posterior parts. Hens treated the breast region of the plumage mainly, whereas during later oilings within an oiling bout, hens tended to frequent other regions such as the wings and the flanks as well (fig. 1, fig. 2). Even within one oiling such a patterning was observed, as the breast was likely to be stroked or rubbed first and/or mainly (fig. 1). This differentiation in oiling the plumage with most attention to the breast part has also been observed in gulls (van Rhijn 1977) and several species of Anatidae (McKinney 1965), and suggests that uropygial gland lipids are specifically functional for the breast part of the plumage. As the back and the tail region were oiled seldom, an experiment in which hens are prevented to oil different parts of the plumage such as the breast and the back (e.g. by using special collars) would elucidate the specific functional aspects of uropygial gland oil.

The localities of the plumage that did receive oil from the uropygial gland, were treated in rather swift and irregular movements. Further micro-morphological analysis of (breast) feathers that are sampled immediately after oiling or subsequent preening should verify how the oil gland lipids are distributed over the individual feathers. Ishida et al. (1973) found an irregular distribution of gland lipids on the distal parts of rooster feathers, but it is not sure whether they sampled the feathers immediately after oiling behaviour. Moreover, these birds had been beak-trimmed. Both the deformation of the beak and reduction in functional sensory input (Breward & Gentle 1985) might have caused an abnormal distribution of the lipids.

An irregular oiling-like distribution of lipids on the feathers of the breast was adopted in testing the causality of feather lipid condition on dustbathing behaviour. As this procedure mimics a natural distribution of the lipids, whereas Borchelt et al. (1979) sprayed lipids over the plumage, a comparison in effectiveness of both methods would be useful, if performed in one study. Application of the first method with stale uropygial gland lipids within a three day period of sand deprivation, caused hens to dustbathe significantly longer after deprivation compared to a fresh lipid or a control treatment (table I), while within the dustbath the total numbers of the side rubbing and side lying elements were strongly enhanced only (fig. 3). The latter is of special interest as specifically side lying and side rubbing act consummatory; they are always preceded by preparatory tossing activities (Borchelt 1975; Vestergaard et al. 1990; Chapter 2), and intensify the contact between the feathers and dust particles, that subsequently adsorb the feather lipids (Healy & Thomas 1973; Borchelt & Duncan 1974; Chapter 2). Thus, it can be concluded that during a period without adequate dustbathing material, a change in quality of the lipids that have been dressed on the feathers, is likely to enhance hen's motivation to dustbathe. In contrast, fresh lipids that had been applied on the breast feathers, did not affect the duration of the dustbath (table I), while the effects on side rubbing and side lying were not straight forward (fig. 3). Fresh lipids oiled on the feathers, therefore, do not promote dustbathing behaviour extra after dust deprivation. Although the strain or age of the hens that provided the treatment lipids, could have affected the results, it does not seem efficient that hens respond to fresh feather lipids per se, if they are functional for the integument, as Elder (1954) suggested. However, if stale lipids are not or less functional, then responding to stale feather lipids is of biological interest. In nature, or semi-natural environments, fowl may postpone dustbathing e.g. when the soil is wet and unattractive for dustbathing (Hein 1970; Huber 1987), in winter time (Fölsch 1981; Huber 1987), when the environmental temperature is low (Klinger 1985), or perhaps during incubation of the eggs, while the dustbathing frequency differs strongly between individuals (Vestergaard 1982; Huber 1987). As a consequence, hens delay the removal of (old) feather lipids, which are thus likely to vary in quality. Therefore, feather lipid quality may interact at different levels on the motivation to dustbathe with other factors, such as those associated with the illustrated conditions (e.g. lighting factors: Huber 1987). An examination of the causal significance of lipids that are distributed over the (breast) feathers after exposure to air at variable lengths of time should elucidate this.

## ACKNOWLEDGEMENTS

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## Chapter 4

# EFFECTS OF LONG-TERM DEPRIVATION OF SAND ON DUSTBATHING BEHAVIOUR IN LAYING HENS

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# EFFECTS OF LONG-TERM DEPRIVATION OF SAND ON DUSTBATHING BEHAVIOUR IN LAYING HENS

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## ABSTRACT.

During 21 weeks of sand deprivation, intact and beak-trimmed laying hens *Gallus gallus domesticus*, dustbathed on a barren floor (sham-dustbathing). The amount of dustbathing increased during the experiment to the same level (in the intact hens) as in non-deprived control hens, or to a higher level (in the beak-trimmed hens). During deprivation, the proportion of complete sham-dustbaths increased, while the consummatory rubbing behaviour within these sham-dustbaths seemed to become more dominant than in baths in sand. After 16 weeks deprivation, abnormal sham-dustbaths started with rubbing instead of appetitive tossing behaviour. The hypothesis that the motivation to dustbathe increases during deprivation was supported, but on the first day after the long-term deprivation, there was no significant compensation for the deprived bathing in sand, and instead signs of conflict bathing and fear were found. Thus, sand as a bathing material becomes unfamiliar after long-term deprivation. In a second experiment, intact hens were deprived of sand for up to 30 weeks, but at either 3-5 or 28-30 weeks they were prevented from sham-dustbathing for 3 consecutive days. As they compensated for the deprived sham-dustbathing activity thereafter, the performance of dustbathing per se may be facilitated intrinsically.

## INTRODUCTION

Galliforme birds perform dustbathing behaviour at regular intervals, which is thought to maintain the amount and quality of the feather lipids and the structure of the feathers (Healy & Thomas 1973; Borchelt & Duncan 1974; Levine et al. 1974; Chapter 1, 2 and 3). When chickens are deprived of dustbathing material (sand), high levels of lipids develop on their feathers, while the lipid quality alters. Enhanced levels of stale feather lipids facilitate bathing behaviour (Chapter 3), and when deprived chickens are given sand again, they increase their bathing behaviour. Dustbaths are performed frequently and for long periods, while excessive lipids are removed (Chapter 1 and 2). For both Bobwhite quail (*Colinus virginianus*), and hens, the duration of the deprivation is thought to correlate positively with the amount of subsequent bathing behaviour (Borchelt et al. 1973; Borchelt 1975; Vestergaard 1980), which supports the hypothesis that deprivation of litter enhances the motivation to dustbathe (cf. Dawkins 1988). McFarland (1989), however, suggested another possibility: the motivation to perform the deprived activity decreases during the deprivation period due to habituation, while the rebound in dustbathing after deprivation of dust is due to dishabituation. Although the terms habituation and dishabituation have a descriptive, not an explanatory significance, and habituation during absence of a stimulus conflicts with its general definition (Kandel 1976; Dickinson 1980; MacKintosh 1983; Staddon 1983; Gould & Marler 1984; Hollis 1984) McFarland's hypothesis is an interesting alternative. We tested both ideas by examining how much dustbathing hens performed during 5 months of sand deprivation (hens dustbathe even though no litter is present: Black & Hughes 1974; Martin 1975; Vestergaard 1980; Bessei & Klinger 1982; Vestergaard et al. 1990; throughout this paper, baths without litter are called sham-dustbaths). We also investigated the dustbathing response of the hens immediately after, and for 6 weeks following the deprivation, as McFarland (1989) stressed the importance of novelty of the stimulation after deprivation. This is of interest, as a long-term deprivation may bring about such a large discrepancy between the perceived situation when litter is reintroduced and the familiar environment without litter, that hens may show conflict or fear (cf. Sokolov 1960; Hinde 1970; Levine et al. 1989) instead of dustbathing.

Bathing in sand has both appetitive and consummatory sequences. The first (called tossing behaviour hereafter) with elements such as vertical wing shaking, or scratching with one leg (see Methods), raises litter onto the plumage, and is performed with fluffed feathers, which facilitates the penetration of particles. The second (called rubbing behaviour), which includes lying on and rubbing the sides of the body, is always preceded by tossing behaviour, in hens (Vestergaard et al. 1990; Chapter 2), as well as Bobwhite quail (Borchelt 1975), and is performed with flattened feathers, which intensifies the contact between the particles, which have been tossed between the feathers, and the integument. This suggests that the first behaviour patterns prepares conditions for the second bathing activity (Chapter 2), but it is not known how both components become organized when litter stimulation is lacking for a long time. Therefore, we also observed how the sham-dustbath is performed. The performance of sham-dustbathing per se may have causal significance (Glickmann & Schiff 1967; Herrnstein 1977). Reviewing the neurophysiological literature, Glickman & Schiff (1967) suggested that facilitation of a species-specific behavioural pattern, and specifically the neural systems, which mediate consummatory acts, would be sufficient for reinforcement. Herrnstein (1977) mentioned autoshaping of e.g. the pecking response by pigeons (*Columba livia*) in Skinner boxes, to illustrate that responses are not hedonically neutral. These ideas owe much to Lorenz (1981), who explained the occurrence of vacuum activities by a damming up of the motivation, resulting from a production of action specific excitability of the motor pattern itself. Although Lorenz's ideas have received much criticism, the basic notion that a behavioural programme may promote itself is significant, as such a reinforcement seems functional in terms of developing and maintaining sensory, neuronal and musculature parts, necessary for that programme. With this line of reasoning, it is possible that the effects of bathing per se are experienced. These are likely to be beneficial and to promote the behaviour itself, if hens sham-dustbathe more to compensate for a short-term lack of it. In a second experiment we tested this by preventing hens from sham-dustbathing, while monitoring its occurrence before and after short-term sand deprivation (3-5 weeks) and long-term sand deprivation (28-30 weeks).

Since the effects of long-term deprivation of litter may have significance for the laying hen industry, which is commonly practised with beak-trimmed hens, the first experiment was performed with intact as well as beak-trimmed hens. The relation between beak-trimming and dustbathing behaviour is not clear, but amputation of the bill does affect other feather maintenance activities such as preening behaviour (Duncan et al. 1989; Craig & Lee 1990).

## EXPERIMENT I

### *Animals, material and methods*

#### *Animals and housing*

We performed the experiment twice in 2 years, each trial starting in October. In one trial the bills of the hens were left intact, while in the other trial the bills were trimmed in line with standard poultry industry practice with a cauterizing blade at the age of 6 weeks: half of the upper and one third of the lower mandible was removed, respectively. All other conditions were comparable for both trials. In each trial 32 Warren laying hens were obtained at the age of 18 weeks from a commercial dealer. From hatching onwards, they had been kept in wire cages without any litter, and with food accessible for feeding only and not for dustbathing. On their arrival, the hens were divided into two groups and housed on wood-shavings. Each (260 x 240 x 220 $cm^3$; l x w x h) pen included two trays of 60 x 60 x 12 $cm^3$, which were filled with river sand and refilled twice a week. At the age of 23 weeks the hens were at random individually housed in wooden cages, measuring 100 x 50 x 50 $cm^3$. The floor of the cage consisted of wooden slats of 50 x 50 $cm^2$, which had 2 cm of space in between, and a dust box of 50 x 50 $cm^2$, which included a layer of 15 cm of dry river sand. This sand was refreshed once a week. Opposite to the dust box there was a nestbox of 39 x 30 x 40 $cm^3$ with a floor of artificial grass. The cages were placed side by side in two opposing rows. Although openings in the cages permitted tactile, auditory and visual contact, tactile contact with the dust or dust box from the neighbouring cage was not possible. Moreover, sand from one cage could not litter the other. Food and water were provided ad libitum from containers that opened adjacent to the nestbox. Twice a day, droppings were sifted out of the sand or scraped from the wooden bottom of the bin when sand was not present (see below). The hens were maintained on a 16:8 h light and dark cycle (lights on at 0600 hours). Daily temperature varied between 14 and 27 or 14 and 24°C for the intact and the beak-trimmed group, respectively, and averaged for both 21°C. Humidity averaged 44% (min. 35%, max. 65%) and 50% (min. 33%, max. 75%), respectively. After 2 weeks to allow the hens to adjust to the new environment, the experiment started. Test hen cages alternated with the control hen cages.

*Design and observations*

Each trial lasting 29 weeks was divided into three periods: a pre-deprivation period of 2 weeks with sand in the dust box (week 1 and 2), a deprivation period of 21 weeks (weeks 3-23), in which all sand was removed from the dust box of the test hens, and a post-deprivation period of 6 weeks (week 24-29), with the dust box again filled with sand. The control hens had access to sand in their bins during the whole trial. Time lapse video recordings were made of 10 test and 10 control hens. (The remaining hens were not observed, but all hens contributed in monitoring plumage condition as part of another test related to dust deprivation). The test hens were recorded in week 1 (prior to deprivation), weeks 3, 5, 7, 10, 17, 23 (while deprived of sand) and weeks 24, 27 and 29 (after deprivation) and the control hens in weeks 2, 4, 6, 8, 11, 18, 22 and 28. Within these weeks each test or control hen was recorded for one day. Dustbathing was observed from the videotapes with the different days and weeks in random order. Bathing in sand or sham-dustbathing was considered to start whenever a hen squatted down in the sand, on the barren floor of the dust box, or on the wooden slats of the cage and performed a vertical wing shake (see below). The end of a bath in sand or a sham-dustbath on a barren floor was determined by the moment a hen stood straight up again or by the start of an interval of at least 5 min, in which the hen, while lying down, did not perform any dustbathing behaviour. Shorter intervals were considered to belong to one uninterrupted dustbath.
Dustbathing behaviour consisted of the following.

(1) Vertical wing shaking: the hen lies on her breast with her feathers fluffed and extends one or both wings laterally, immediately followed by rhythmic movements of the legs, which scratch the litter vertically upwards (and so toss litter between the trunk and the wings onto the plumage), or which scratch along the barren floor.

(2) Scratching with one leg: the hen lies on the ventrolateral part of the trunk with the feathers fluffed and scratches the contra-lateral leg several times in the litter (and so tosses litter onto the plumage) or along the floor.

(3) Bill raking: the hen points her closed bill away from the body in the litter or to the bottom of the cage and moves her bill through the litter or along the bottom, respectively, towards the body.

(4) Head rubbing: the hen lies on a lateral part of the trunk and fluffs the feathers of her neck, while rotating and moving the ipsilateral part of the head against the litter or along the bottom of the cage in a distal-proximal direction.

(5) Side lying: the hen lies on the (dorso)lateral part of the trunk, while flattening the feathers and keeping the wings tightly against the body, and may orient the head in a dorsal direction.

(6) Side rubbing: the hen lies on her side (5), but now one or both legs is stretched against the rim of the dustbathing hole in the substrate or against the wooden bottom or walls of the dust box, which results in the hen partly rotating along her longitudinal axis.

### *Statistics*

The descriptive parameters were medians and third quartile deviations (median-third quartile ranges), calculated for each week, within each treatment, respectively, since distributions of the variables were unknown. For multiple repeated measurement, or pair-wise comparisons within the control or the test hens, a Friedman one way analysis of variance, and a Wilcoxon signed-ranks matched-pair test was used, respectively. For comparisons between control and test hens a Wilcoxon independent sample test was used (Conover 1980). All statistical comparisons were two-tailed.

### *Results*

The duration and daily frequency of the dustbaths performed by both the intact and the beak-trimmed control hens did not change significantly over the whole experimental period (figs. 1 and 2). Prior to deprivation, both the duration and the daily frequency of the dustbaths of the intact and the beak-trimmed test hens did not differ significantly from those of the control hens.

In the first week of deprivation (week 3) fewer intact and beak-trimmed test hens dustbathed (fig. 1). As a consequence, bath duration was estimated on the basis of a small sample size, and could not be tested statistically. However, a significant decrease in daily dustbath frequency was found, for both the intact and the beak-trimmed hens (fig. 2; $P< 0.01$ and $P< 0.05$, respectively).

Over the weeks of deprivation, the durations of the sham-dustbaths performed by both the intact and the beak-trimmed hens seemed to increase (fig. 1). Owing to a small number of sham-dustbathing hens early in the deprivation, and an inconsistent patterning over time of the number of animals that did sham-dustbathe, statistical verification of this increase was not possible. However, the daily frequency of sham-dustbathing over the deprivation period increased significantly, for both the intact and the beak-trimmed hens (fig. 2; $P< 0.01$ and $P< 0.05$, respectively). The median daily total time spent sham-dustbathing (with third quartile deviation) increased gradually during the deprivation from 0 (0) and 0 (1.2) min. in week 3, to 16.7 (11.6) and 39.8 (17.1) min. in week 23 for the intact and the beak-trimmed hens, , respectively ($P< 0.01$, in both cases). For the control hens, no significant change was found, and overall daily total time of dustbathing was 17.0 (4.4) and 15.9 (10.5) min., respectively.

At the end of the deprivation (week 23), the sham-dustbath duration did not differ significantly from the duration of the sand baths of the control hens (week 22) of both the intact and the beak-trimmed hens (fig. 1). Daily frequency of dustbathing and total time spent dustbathing per day also did not differ for these weeks between intact test and control hens (fig. 2). For the beak-trimmed test hens, however, the levels of the latter parameters were significantly higher than for the control hens ($P< 0.05$ in both cases).

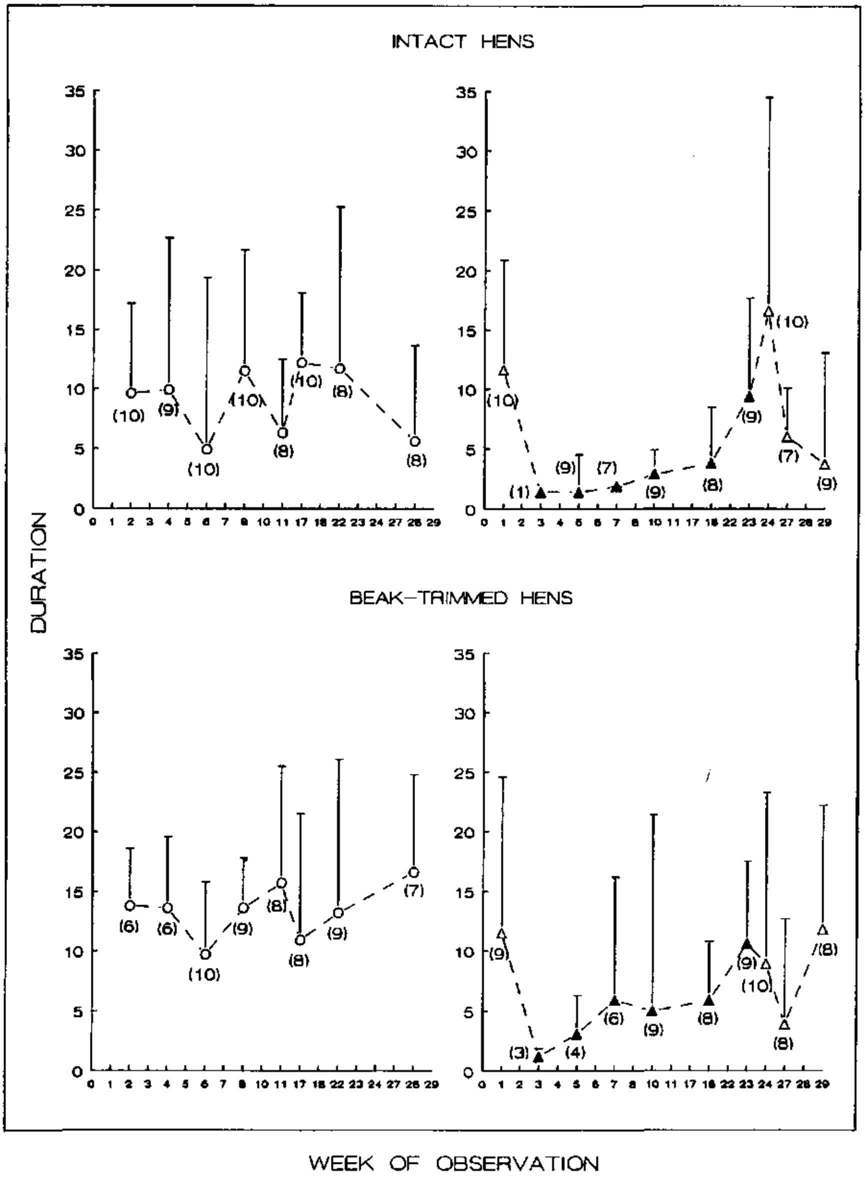

Figure 1. Median duration (with third quartile deviation; min.) of dustbaths performed by intact and of beak-trimmed hens that had sand in their cage continuously (controls: ○) or had sand before week 3 and after week 23 (test hens: △ sand in the cage; ▲ no sand in the cage). Number of dustbathing hens is given. See the text for statistical comparisons.

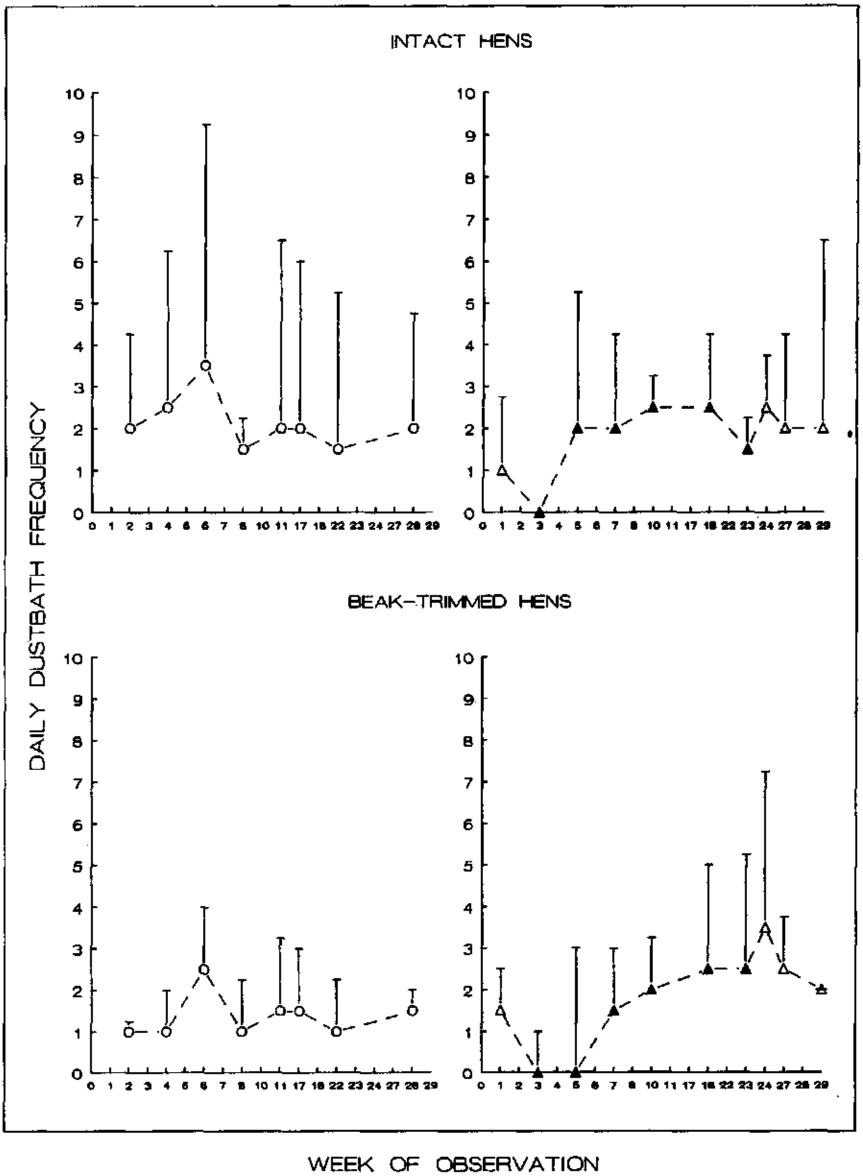

Figure 2. Median daily dustbath frequency (with third quartile deviation) performed by intact and beak-trimmed hens that had sand in their cage continuously (controls: ○) or had sand before week 3 and after week 23 (test hens: △sand in the cage; ▲no sand in the cage). See the text for statistical comparisons.

Until weeks 5-7 of the deprivation period, only short sham-dustbaths that included only bill raking, head rubbing, scratching with one leg and/or vertical wing shaking were performed (these were defined as incomplete baths). Thereafter, side lying and side rubbing were added to the sham-dustbathing sequences (a complete bath was defined as one that includes all elements, irrespective of their number). The median ratio of the number of complete baths in the total number of sham-dustbaths was 0.00 and 0.03 over the first weeks of the deprivation period (week 3, 5 and 7), but 0.50 and 0.43 at week 23 ($P < 0.05$ in both cases), which did not differ significantly from the overall control level of 0.50 and 0.61 for the intact and the beak-trimmed hens, respectively. However, at week 23, both the intact and the beak-trimmed hens performed significantly fewer vertical wing shakes, and took significantly less time preceding the first rubbing behaviour, than did the control hens in their sand baths (table I; $P < 0.01$ and $P < 0.05$, respectively, in both "bill" treatments).

Table I. Median total number of vertical wing shakes, and latency in minutes (with third quartile deviation) until the first rubbing behaviour in complete dustbaths performed by intact or beak-trimmed hens that had continuous access to sand (CONTROL), or had access before and after a deprivation from week 3 to week 23 (TEST).

| | | BEFORE DEPRIVATION | AT END OF DEPRIVATION | AFTER DEPRIVATION | |
|---|---|---|---|---|---|
| | | WEEK 1/2 | WEEK 22/23 | WEEK 22/24 | WEEK 28/29 |
| **INTACT HENS** | | | | | |
| WING SHAKES | CONTROL | 15 (4) | 22 (1) | 22 (1) | 14 (4) |
| | TEST | 16 (3) | 6 (5)** | 19 (8) | 18 (1) |
| LATENCY | CONTROL | 6.2 (1.7) | 9.3 (2.6) | 9.3 (2.6) | 5.4 (1.4) |
| | TEST | 6.1 (4.1) | 3.6 (4.0)* | 13.9 (4.4) | 6.0 (1.6) |
| **BEAK-TRIMMED HENS** | | | | | |
| WING SHAKES | CONTROL | 12.5 (4) | 17.5 (5.5) | 17.5 (5.5) | 17 (9) |
| | TEST | 14 (2) | 5.5 (2)** | 15 (7) | 14.5 (7.5) |
| LATENCY | CONTROL | 6.0 (1.7) | 6.2 (2.9) | 6.2 (2.9) | 8.3 (0.4) |
| | TEST | 6.1 (1.5) | 2.8 (2.5)* | 6.9 (3.9) | 7.3 (3.9) |

Control-test comparisons: *: $P < 0.05$; **: $P < 0.01$.

Before as well as after the deprivation period, these levels did not differ significantly. Moreover, at weeks 18-23, sham-dustbaths were performed, that started not with tossing, but with rubbing behaviour, while some consisted of rubbing behaviour only. Owing to the way dustbaths were defined a priori, we did not document them systematically.

At renewed access to sand (week 24), all test hens immediately explored the sand, by scratching with two legs, while pecking at or into it. Dustbathing always occurred thereafter, and its duration and daily frequency did not differ significantly from the levels of the week before, both in the intact and the beak-trimmed hens (fig. 1 and 2). Moreover, the week-24 levels did not differ from those of week 22 of the control hens, except again for the daily dustbathing frequency of the beak-trimmed birds, which was significantly higher than in the control hens. However, two of the 10 intact hens and three of the 10 beak-trimmed hens did not start a sand bath, but performed a sham-dustbath on the wooden slats of the cage, while bill-raking in the sand. Hens that did bathe in the sand performed incomplete scratches with one leg and incomplete vertical wing shakes, while their succession appeared to slow down (cf. table I by comparing the number of wing shakes per latency minute in week 22/24). In addition, 74% (median) of the dustbaths (third quartile deviation: 26%; number of baths: see fig. 2) performed by the beak-trimmed hens after the long-term deprivation (week 24) ended with the hen immediately fleeing from the sand, which was significantly more frequent than in the control hens in week 22 (0%; third quartile deviation: 33%; $P < 0.05$). For the intact hens this difference was not significant: the median value was zero both for the test and the control hens, although the third quartile deviations were 62% and 23%, respectively.

At the end of the post-deprivation period (week 29), the duration of the dustbaths performed by the intact test hens decreased significantly compared to the previous level of week 24 (fig. 1; $P < 0.05$), while the daily frequency of dustbathing did not change significantly. For the beak-trimmed test hens, no significant change in duration was observed, although a significant decrease was found in the daily frequency of the dustbaths (fig. 2; $P < 0.05$). The duration as well as daily frequency of the dustbaths of the control hens in week 28 did not differ from the week 29 levels of the intact and the beak-trimmed test birds, respectively.

## EXPERIMENT II

### *Animals, materials and methods*

#### *Animals and housing*

For the second experiment, 30 intact Warren hens were purchased, treated and housed in the same way and at the same age as in the first experiment. The period of acclimatizing to the cage with sand, in which the hens were individually housed was 4 weeks. Temperature averaged 23°C (min. 16°C, max. 25°C) during treatment of the first, and 22°C (min. 17°C , max. 23°C) during treatment of the second test group (see below). Humidity averaged 67% (min. 53%, max. 80%) and 48% (min. 40%, max. 55%), respectively.

#### *Design and observations*

The experiment lasted 30 weeks and started after the period of acclimatization by depriving all hens of sand. Within this period of deprivation, two groups, of 10 hens each, underwent a test treatment either at 3-5 weeks, or at 28-30 weeks of deprivation (test hens). The remaining 10 hens received a control treatment (control hens). The test treatment, from now on called frame treatment, consisted of the bottom of the dust box of the cage, the wooden slats of the cage floor and the nestbox floor being replaced by three wooden frames for 3 days. Prior to the exchange the hen was removed temporarily from her cage, and put into a plastic box, which did not contain any litter. Two frames with four transverse sticks (2.8 cm thick and 50 cm long, 7.5 cm open space in between), and a third with three sticks (30 cm long), were placed centrally in both compartments of the cage and the nestbox, respectively, which prevented the hen from lying down, and sham-dustbathing, as no solid surface was present in the cage (fig. 3).

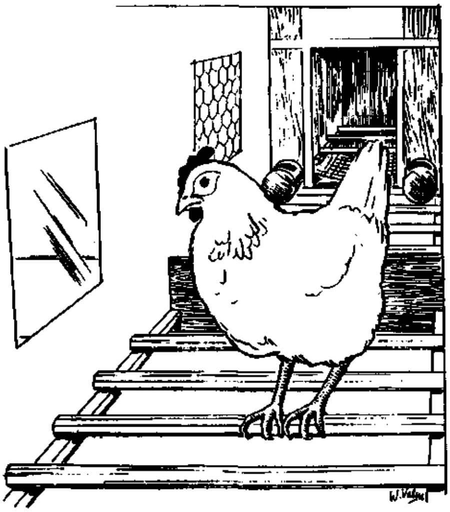

**Figure 3.** Cage with frames of transverse sticks as flooring, which prevents a hen from lying down and dustbathing.

After installing the frames, the hen was put back in her own cage. Pilot observations had confirmed that hens were able to walk over the sticks e.g. to get food and water. At the end of this treatment, the original solid and slat bottoms were reinstalled in the cage. Cages were altered between 1030 and 1100 hours. Each frame treatment was paired with one control treatment. A control hen was taken out and put back into her cage at the same time, and in the same way as a test hen was, but her cage was not altered. The latter hens served as controls both in weeks 3-5 and in weeks 28-30 of the deprivation period.

One day prior to and the day following each pair of frame and control treatments, whole day video recordings were made of the test and the control hen. Observations from these recordings were called the "before" and "after" observations. Sham-dustbathing behaviour was observed in the same way as in the first experiment. After the frame treatment, the latency was monitored between the moment of putting a hen back in her cage until the start of the first sham-dustbathing. Lying behaviour on the bottom of the dust box was observed over the whole day. Lying started the moment the hen's breast touched the bottom of the dust box and ended as soon the hen stood straight up or started a sham-dustbath.

*Results*

Prior to sand deprivation, all hens performed dustbathing in their sand box. During sand deprivation, all sham-dustbathing occurred on the wooden solid bottom of the empty dust box.

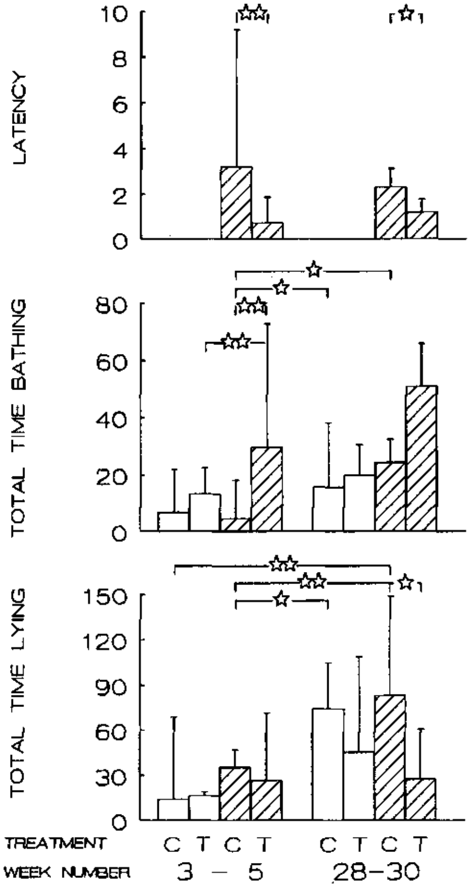

Figure 4. Median latency to intitiate sham-dustbathing (x $10^2$), daily total time spent sham-dustbathing, and daily total time spent lying (with third quartile deviation; min.) at 3-5 and 28-30 weeks of sand deprivation. Per period, 10 control hens, which had no change in their cage (C), and 10 test hens, which had the solid cage bottom exchanged for 3 days for a frame of slats (T) were observed before (open bars) and after (hatched bars) the exchange. *: $P < 0.05$; **: $P < 0.01$.

After the frame treatment, a test hen took significantly less time to start sham-dustbathing than a control hen did, both at 3-5 weeks and 28-30 weeks of deprivation (fig. 4; $P < 0.01$ and $P < 0.05$, respectively). Between weeks 3-5 and 28-30, no significant change in this latency was found; for either the control or the test hens.

Thwarting of sham-dustbathing in deprivation weeks 3-5 resulted in a significant increase in the daily total time spent sham-dustbathing by the test hens, which was also significantly higher than the "after" control level (fig. 4; $P < 0.01$ in both comparisons). At prolonged deprivation (28-30 weeks), the daily total time spent sham-dustbathing did not change significantly in the frame treatment, which was due to zero scores by two hens, which did not lie down at all in the "after" observations, whereas all (8) other hens increased their time spent sham-dustbathing compared to the "before" observations. The total time spent sham-dustbathing by the control hens was significantly higher at prolonged deprivation, compared to early in the deprivation, as shown by comparison between the "before" observations in weeks 28-30, and the "after" ones in weeks 3-5 (fig. 4; $P < 0.05$).

The frame treatment did not significantly affect the daily total time spent lying in weeks 3-5, but significantly reduced this time in weeks 28-30 compared to the "before" levels (fig. 4; $P < 0.05$). In the course of deprivation, daily total time spent lying increased significantly, as shown by three out of the four possible comparisons between weeks 3-5 and weeks 28-30 within the control hens (fig. 4; $P < 0.05$ or $P < 0.01$). The probability that hens continued their lying with sham-dustbathing did not change in the frame treatment, nor during deprivation, and ranged between 20 and 50%.

## GENERAL DISCUSSION

This study supports the hypothesis that the motivation to dustbathe increases during deprivation of dust. The daily frequency (fig. 2) and the total time spent sham-dustbathing each day increased significantly, while duration of the baths tended to increase during deprivation of sand (fig. 1), irrespective of whether hens had intact or amputated bills (for the daily total time of dustbaths see also fig. 4). Sham-dustbaths progressively included more rubbing behaviour during deprivation, which indicates an increase in the motivation to dustbathe, as rubbing occurs only after tossing behaviour (Vestergaard et al. 1990; Chapter 2), and therefore has a relatively high threshold. In addition, during the performance of the complete sham-dustbaths, the consummatory act of rubbing seemed to become more dominant. This is suggested by a reduction in the amount of tossing behaviour, expressed by the number of vertical wing shakes, as well as in the time preceding the first rubbing behaviour, compared to the control level (table I). Moreover, abnormal dustbaths were performed; these started with rubbing behaviour, and could consist of rubbing behaviour only. Vestergaard et al. (1990), however, suggested that there was no difference in motor patterning between sand baths and sham-dustbaths, and did not report any abnormal sequences. Therefore, a more detailed study of the different types of dustbaths at subsequent phases of sand deprivation is needed.

At renewed access to sand after a long-term deprivation, sand can be unfamiliar as a bathing material. Both the intact and the beak-trimmed test hens did not show significantly more or longer dustbaths compared to the level before the sand introduction and/or the control level (figs. 1 and 2). Instead, they showed signs of conflict bathing and of fleeing the sand bath. These findings contrast with those after short-term deprivation (Vestergaard 1980; Chapter 1 and 2), and therefore imply that the level of novelty of the sand, correlated with the duration of the deprivation, determines whether a rebound (McFarland 1989), or a fear response will be performed. This is analogous to an increase in stress, as measured in rats, *Rattus norvegicus*, at increasing rates of unfamiliarity (Levine et al. 1989). However, in the last week of the post deprivation period, the dustbathing responses seemed to have normalized as no significant differences were found for all dustbathing parameters for both the intact and the beak-trimmed hens (figs. 1 and 2, table I). Thus, continued experience with the effects of the sand is necessary to develop and maintain a specific causal link between sand and dustbathing behaviour. This has also been suggested in an ontogenetic context (Vestergaard et al. 1990).

In line with the supposed regulatory function of dustbathing in maintaining feather condition (see Introduction), an increase in the motivation to dustbathe during deprivation can be explained by changes in the integument. Simultaneously, an intrinsic facilitation of bathing behaviour is possible, as indicated by the results of the second experiment. In weeks 3-5 of the deprivation, the latency to start with the sham-dustbathing was significantly reduced, while the daily total time spent dustbathing was significantly higher after a short-term prevention of sham-dustbathing compared to control levels. In the weeks 28-30 of the deprivation, comparable results were found, but two hens showed a divergent response, as they were never seen to lie down after the frame treatment. Why this was the case is not clear, but could be related to individual differences in exploration or anxiety levels expressed after gross changes in a cage, which has been barren for a long time. Individual-dependent responses to environmental changes have been shown in mice, *Mus domesticus*, and rats by Benus et al. (1987), and may resemble strain-dependent responses to frightening stimuli in fowl (Duncan & Fishie 1979). Thus, Glickman & Schiff's (1967), Herrnstein's (1977) and Lorenz's (1981) suggestion that a behavioural programme can be facilitated intrinsically, may be correct, and it is of special interest that Glickman & Schiff as well as Lorenz have marked the consummatory act for such a facilitation. If this is correct, then it may be a matter of neural organization of the dustbathing behaviour, to explain why the consummatory act within the sham-dustbath (rubbing behaviour) seems to become more pronounced in the course of the deprivation "at the cost" of the expression of the appetitive tossing part. As suggested earlier we need to know more about the development of sham-dustbathing, but it would be intriguing to know whether there is a neurobiological basis for differentiating between tossing and rubbing behaviour.

Lying behaviour increased in the course of the deprivation (fig. 4), which may suggest that postural facilitation plays a role in the occurrence of bathing. However, the first experiment showed that the motivation to dustbath itself is involved, which supports the reversed reasoning. After the frame treatment, the enhanced bathing response was not related to the tendency to lie down, as the amount of lying did not differ, or was significantly less than before the frame treatment (fig. 4), while the probability that lying was continued with sham-dustbathing did not change significantly.

The general impact of long-term sand deprivation on bathing was comparable between the beak-trimmed and the intact hens. However, both "bill" treatments differed in some specific responses. To account for these in terms of the bill condition only, would not be valid, as other (unknown) systematic differences between both trials of the first experiment may have occurred. Instead, it would be of interest to include both "bill" treatments within one trial.

In conclusion, both the amount of dustbathing, and the proportion of complete dustbaths increased during deprivation of dust. Moreover, within the complete sham-dustbath the occurrence of the consummatory rubbing behaviour seemed to become more dominant, while prevention of the sham-dustbath performance resulted in compensation for the deprived sham-activity afterwards. Thus, during deprivation the motivation to dustbathe increases, which, together with conditional changes in the integument, may have resulted from an intrinsic facilitation. Instead of intensive bathing behaviour at renewed access to sand, conflict behaviour and fear were elicited, which implies that experience is needed to maintain the use of sand as a bathing material.

## ACKNOWLEDGEMENTS

We thank Hans Romberg for his assistance in programs and computers, Françoise Wemelsfelder for helpful discussions about long-term habituation and Wim Valen for his excellent drawing of a hen on sticks.

## Chapter 5

# DUSTBATHING AND ITS EFFECT ON PROXIMAL AND DISTAL FEATHER LIPIDS IN LAYING HENS

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# DUSTBATHING AND ITS EFFECT ON PROXIMAL AND DISTAL FEATHER LIPIDS IN LAYING HENS

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## ABSTRACT

After prolonged experience with wood-shavings or with sand 2 x 11 hens (*Gallus gallus domesticus*) were litter deprived. Prior to the 7 to 8 day deprivation, immediately hereafter and following the first bath in the familiar litter, feather samples were taken. Lipids were extracted from sample subdivisions which contained either proximal plumulous or distal pennaceous parts. Hens on wood-shavings bathed 10 min. longer than hens on sand. This was due only to an extension of the bathing phase, which included rubbing; the preceding phase of tossing did not differ. In the extended phase of the wood-shavings bath the tendency to rub was lower, whereas the tendency to toss was higher than in the comparable phase of the sand bath. Thus, litter quality affected the amount of rubbing and subsequent tossing. Rubbing did not effectuate a close contact between wood-shavings and the proximal integument, as wood-shavings could not be tossed into the plumage. This contrasted baths in sand and only these baths resulted in a removal of excessive lipids from the plumulous parts. In this respect rubbing functionally seems crucial. The lipid level immediately after deprivation and the change in the amount of lipids due to deprivation positively correlated to the amount of tossing in wood-shavings and to the amount of rubbing in sand. This indicates that the lipid condition is causally involved in dustbathing.

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## INTRODUCTION

Dustbathing in fowl is functionally organized in sequences of tossing and rubbing behaviour. Tossing, such as vertical wing shaking, is performed with the feathers fluffed. Rubbing includes side rubbing and is performed with the feathers flattened and the wings kept firmly to the body (cf. Kruijt 1964; Borchelt 1975; Fölsch 1981; Klinger 1985). Tossing behaviour serves the distribution of the litter over and into the plumage and always precedes rubbing (in quail: Borchelt 1975; in hens: Vestergaard et al. 1990; Chapter 2). Rubbing intensifies the contact between the feathers and the litter, which has penetrated the plumage. Physical properties of the litter affect this penetration: sand easily reaches up to the skin after tossing behaviour, whereas wood-shavings do not and adhere to the distal parts of the plumage. Simultaneously, tossing seemed to be more frequently reinitiated following rubbing behaviour in wood-shavings baths than in sand baths (Chapter 2). This indicates that the litter quality affects the organization of a bath which may correspond to the effect of litter on the plumage. Feathers are dressed with skin lipids and uropygial gland lipids (Lucas & Stettenheim 1972b; Ishida et al. 1973; Hodges

1974; Borchelt et al. 1979; Chapter 3) which are thought to be adsorbed and removed by the dust of a bath when they become excessive (Healy & Thomas 1973; Borchelt & Duncan 1974). It is therefore expected that the exact location of the particles is functionally essential during a bath, in particular in reducing the amount of feather lipids. The occurrence of tossing and rubbing behaviour after deprivation of wood-shavings or sand and the efficiency of lipid removal from proximal and distal parts of feathers is investigated in the present study. A differentiation between breast and back feathers is thought appropriate, as oiling behaviour is mainly directed to the breast and hardly to the back (Chapter 3). Moreover, breast feathers contact the litter during lying behaviour and, thus, may be differently affected compared to back feathers.

In addition it is aimed to examine correlations between feather lipid levels and quantities of tossing or rubbing behaviour, respectively. Tossing and rubbing may differently correlate, as specifically the quantity of rubbing (in sand) was enhanced due to an application of stale uropygial gland lipids to the plumage (Chapter 3).

## ANIMALS, MATERIAL AND METHODS

### *Animals and housing*

Twenty-four Warren laying hens with intact beaks were obtained from a commercial dealer at the age of 18 weeks. From hatching onwards they had been reared in wire cages without any litter; moreover, the food had not been accessible for dustbathing. On their arrival the hens were housed on wood-shavings in a 260 x 240 x 220 $cm^3$ (l x w x h) pen. At the age of 33 weeks the hens were wing tagged for individual identification and randomly divided into four groups of six birds. These groups were housed in 150 x 260 x 260 $cm^3$ pens with wooden slatted floors and nest boxes, which did not contain any litter. The pens were placed in a single row and the groups were visually isolated from each other. In the corner of each pen, a 60 x 60 x 12 $cm^3$ tray was enclosed in a 63 x 63 x 50 $cm^3$ wire cage which could be entered through a lockable sliding door. The tray was filled with litter and refilled twice a week. The litter in the tray was sand in the first and third group and wood-shavings in the other two groups. A 14/10 light/dark cycle (lights on at 0600 hours) was maintained. Food from a small feeding trough (which did not permit hens to use food for bathing) and water were provided ad lib.. Air temperature averaged 18°C; average relative air humidity was 55%. The experiment started after 4 weeks of acclimatization. During this period the animals entered the tray and only dustbathed in the provided litter.

### *Experimental design*

The experiment covered ten days. In the afternoon of day one feathers from each hen were individually sampled. From day two to day eight all hens were deprived of dustbathing litter by locking the wire cage that contained the dust tray. On day nine the first three hens and on day ten the second three hens of a group were permitted to dustbathe one after the other (see section Behavioral observations) immediately following a second feather sampling. In the period between the observations of day nine and day ten the wire cage remained locked. In this design the first post-deprivation dustbaths of all hens could be observed between 1300 and 1700 hours which suited the daily distribution of dustbathing behaviour (Vestergaard 1982; Vestergaard et al. 1990). Feathers were sampled for a third time immediately after each hen had dustbathed.

### *Behavioural observations*

After the second sampling of the plumage a hen was put in the tray inside the wire cage which remained closed for the other hens of the group. In this way, each hen was temporarily kept isolated in the litter tray, while only visual and auditory contact was possible with the hens outside the enclosure. As soon as the hen was left in the wire cage, the observations started. Behaviour was continuously recorded with an OS-3 microcomputer (Observational Systems Inc., Seattle, Washington). The start of a dustbath was defined as the first occurrence of vertical wing shaking; the end was marked by body/wing shaking or by non-bathing behaviour if this lasted for more than 5 minutes. Interruptions of less than 5 minutes were considered to belong to the dustbath. Dustbathing behaviour was divided into two phases. Phase 1 was defined to last until the first occurrence of rubbing (see below) and, thus, comprised only tossing (see below) and some non-dustbathing behaviour. Phase 2 was the remaining part of the dustbath, which by definition, included sequences of rubbing together with sequences of tossing and other behaviour. Tossing behaviour was defined as a sequence of vertical wing shaking, bill raking, head rubbing, scratching with one leg or lying, sharing the characteristic of a fluffed plumage. Although bill raking could also be performed while the plumage was not fluffed, it was classified "tossing behaviour", because it occurs within the loop of bill raking, scratching with one leg, head rubbing, vertical wing shaking and bill raking again (cf. Vestergaard 1981, the Introduction of this thesis and for quail Borchelt 1975). Rubbing behaviour was defined as a sequence of side lying or side rubbing which were always performed with the feathers flattened and the wings held tightly to the body. A detailed description of the dustbathing elements is given in Chapter 4. The behaviour's minimum duration to be reliably measured was one second. Sequences either of bill raking, vertical wing shaking or scratching, in which the movements succeeded within one second, were recorded as one bill rake, vertical wing shake or scratch, respectively.

### *Feather sampling and lipid extraction*

Before the litter deprivation, after the deprivation and immediately after the first dustbath following the deprivation two feather samples were taken: one from the breast region and one from the back region of the plumage. Feathers were cut off at their base at eight fixed locations per region, i.e. 5 feathers per location. The locations at the back region were dorsally at the posterior cervical, the interscapular and dorso pelvic tract; those at the breast region were ventrally at the posterior cervical and at the pectoral tract (Lucas & Stettenheim 1972a). Each feather was mature, apparently clean and included a proximal plumulous part and a distal pennaceous part. After sampling, the feather was cut at the transition between the plumulous and pennaceous part, which was recognized by the colour and structure of the barbs (white plumules and brown pennae). Thus, 3 (time of sampling) x 2 (region of the plumage) x 2 (feather part) samples (weighing 0.5-1 g), containing 40 feather parts each, were obtained per hen.

A Soxhlett cold extraction method (Anonymous 1983) was applied in order to extract lipids from the feather part samples. After two hours the solvent (petroleum spirit: boiling range 40-60°C) with lipids was poured from the distillation receiver into a pot of glass. Fresh solvent was used twice to clean the receiver and, subsequently, added to the pot. After distillation of all solvent the pot, containing the lipid residue, was cooled down to room temperature and weighed. The weight of the lipid residue ranged between 2.8 and 11.6 mg. For the purpose of reliably estimating the weight of this residue, the pot, weighing 12 g, suited better than the distillation receiver, weighing 60 g. The amount of extracted lipids was expressed per gram (non-dried) feathers.

### *Statistics*

Total duration of the dustbaths was divided into the duration of phase 1 and the duration of phase 2 (cf. the definition in the section "Behavioural observations"). Per hen, the median duration of each dustbathing element and its total number was calculated. Subsequently, the number of vertical wing shakes in phase 1 (representative for tossing) and of side rubbings in phase 2 (representative for rubbing) were expressed per minute of phase 1 and phase 2, respectively. In addition, this was done for the number of vertical wing shakes in phase 2, which were performed during the intervals between subsequent rubbings (these intervals combine to the so called phase 2 residue). Median duration and total number of sequences, which included either only tossing, rubbing or other behaviour (cf. the section "Behavioural observations") were calculated per hen. These sequences were defined as tossing, rubbing and other behaviour events, respectively.

Medians and third quartile deviations (the median - third quartile range) were calculated per substrate treatment for each of the afore mentioned parameters. Median amount of feather lipids were calculated per part of the feather, region of the plumage, sampling period and substrate treatment. A Wilcoxon independent sample test was used for comparisons between both substrate treatments. A Wilcoxon signed-ranked matched-pair test was used for comparisons within substrate treatments, regions of the plumage and parts of the feather (Conover 1980). Relations between the lipid levels of the different feather parts and between the amount of feather lipids and the total time spent tossing or the total time spent rubbing were examined with a Spearman ranking correlation (Conover 1980) per substrate treatment. One hen in the sand treatment dustbathed for 12 minutes, but did not perform any rubbing behaviour. One hen in the wood-shavings treatment had injured her leg. These hens were excluded from the experiment which, as a consequence, reduced the sample size to 11 per substrate treatment. All tests were two-tailed.

## RESULTS

The latency to start dustbathing after the 7 to 8 days of deprivation did not differ significantly between the substrate treatments (table I). The total duration of the dustbaths, however, was about 10 minutes longer in wood-shavings than in sand (table I; $p < 0.01$). This difference was mainly caused by the duration of phase 2 (table I; $p < 0.05$).

Table I. Several parameters of bathing behaviour in sand or in wood-shavings after a 7 to 8 day litter deprivation. Medians (with third quartile deviations) are given; all duration estimates in minutes ($N = 11$ per treatment).

| | SAND | WOOD-SHAVINGS |
|---|---|---|
| LATENCY | 2.3 (0.7) | 2.1 (1.6) |
| TOTAL DURATION | 29.9 (5.6) | 39.0 (5.7)$^{**}$ |
| DURATION PHASE 1 | 14.4 (4.1) | 12.2 (11.7) |
| DURATION PHASE 2 | 15.4 (3.4) | 24.5 (6.3)$^{*}$ |
| NR. OF V.W.S./ MIN. PHASE 1 | 2.2 (0.6)$^{A}$ | 2.0 (0.5)$^{A}$ |
| NR. OF V.W.S./ MIN. PHASE 2 RES. | 1.3 (0.2)$^{C}$ | 1.2 (0.3)$^{C}$ |
| NR. OF SIDE R./ MIN. PHASE 2 | 2.7 (1.6) | 1.2 (1.3)$^{**}$ |
| DURATION TOSSING EVENT | 1.8 (4.1) | 1.5 (2.4) |
| DURATION RUBBING EVENT | 0.9 (1.0) | 0.5 (0.2)$^{**}$ |
| DURATION NON-DUSTB. INTERRUPTION | 0.1 (0.4) | 0.6 (2.2)$^{(*)}$ |
| NR. OF TOSSING EVENTS | 11 (4) | 15 (10)$^{*}$ |
| NR. OF RUBBING EVENTS | 10 (4) | 13 (8)$^{(*)}$ |
| NR. OF NON-DUSTB. INTERRUPTION | 1 (1) | 2 (1) |

Sand - wood-shavings comparisons: $^{(*)}$: $0.05 < P < 0.10$; $^{*}$: $P < 0.05$; $^{**}$: $P < 0.01$; Within sand or wood-shavings comparisons: $^{A-C}$: $P < 0.01$. PHASE 1: *phase from start until first rubbing behaviour;* PHASE 2: *phase from start of rubbing behaviour until end of dustbath;* PHASE 2 RES.: *phase 2 residue, i.e. phase 2 excluding the time spent rubbing behaviour;* V.W.S.: *vertical wing shake;* SIDE R.: *side rubbing;* NON-DUSTB.: *non-dustbathing.*

The durations of the dustbathing elements did not differ between both substrate treatments. Head rubbing and side rubbing lasted about 1 second, vertical wing shaking and scratching with one leg lasted about 2 seconds and bill raking and lying lasted about 3 seconds. Side lying lasted significantly less in wood-shavings than in sand (8 (1) v. 11 (3) seconds; $P < 0.05$). The total numbers of bill raking, scratching with one leg, vertical wing shaking and lying, however, were significantly higher in baths in wood-shavings compared to those in sand (fig. 1). The total numbers of other bathing elements did not differ between the substrate treatments.

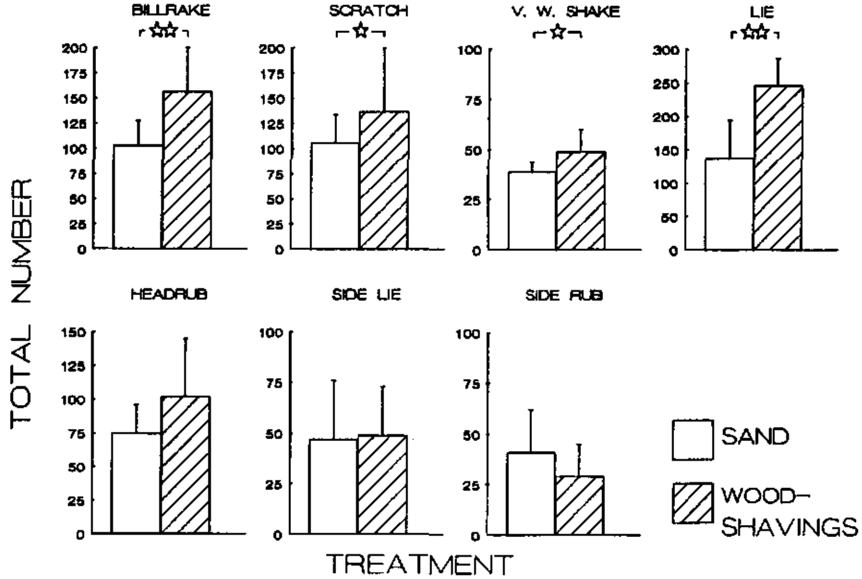

Figure 1. Median total number (with third quartile deviation) of the elements in the first dustbath after a 7 to 8 days period of deprivation of sand or wood-shavings (scratch: scratch with one leg; v.w. shake: vertical wing shake). *: $P < 0.05$; **: $P < 0.01$; $N = 11$ per substrate treatment.

When expressing the number of vertical wing shakes per minute of phase 1 or per minute of the phase 2 residue no significant differences were found between both substrate treatments. However, side rubbing was less frequently expressed per minute of phase 2 in wood-shavings than in sand (table I; $P < 0.01$). Within both substrate treatments, the relative number of vertical wing shakes in the phase 2 residue was significantly lower than in the phase 1 (table I; $P < 0.01$ in both treatments). The duration of tossing events did not differ between the bathing substrates, whereas the duration of the rubbing events was significantly less in wood-shavings than in sand (table I; $P < 0.01$). The number of tossing events was significantly higher in wood-shavings than in sand ($P < 0.05$), while the number of rubbing events tended to be higher, too (table I; $P = 0.09$). The duration of the non-dustbathing events tended to be higher in wood-shavings compared to those in sand (table I; $P = 0.07$), whereas their number did not differ significantly.

The amount of lipids of the plumulous breast feather parts and that of the plumulous back feather parts were significantly higher in the wood-shavings than in the sand treatment prior to the deprivation of litter and after the first dustbath following deprivation (fig. 2). However, these amounts did not differ significantly between substrates immediately after deprivation (fig. 2). The lipid level of the pennaceous parts of the feathers did not show any significant differences between substrates, irrespective of sampling period or of region of the plumage (fig. 2).

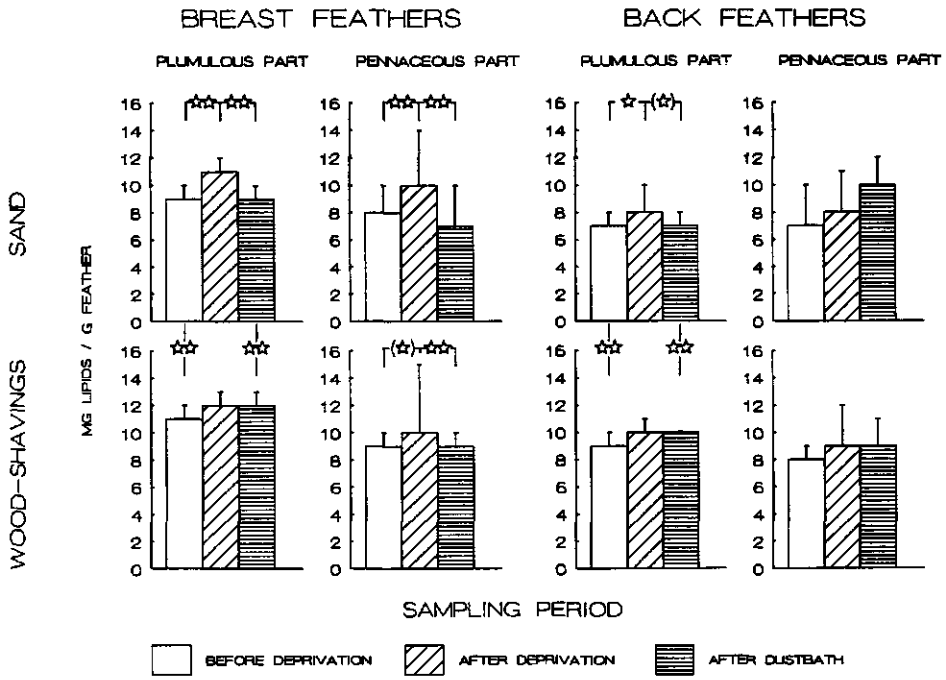

Figure 2. Median amount of lipids (with third quartile deviation) on plumulous and pennaceous parts of breast and back feathers that were sampled before deprivation, immediately after deprivation and after the first dustbath in sand or wood-shavings. (*): $0.05 < P < 0.10$; *: $P < 0.05$; **: $P < 0.01$; $N = 11$ per substrate treatment.

No significant differences in lipid levels were found between the different sampling periods within the wood-shavings treatment, except for the pennaceous parts of the breast. Their lipid level tended to be highest immediately after deprivation, whereas the before deprivation and after dustbathing level did not differ. Within the sand treatment, however, the deprivation significantly enhanced and subsequent bathing significantly reduced the level of lipids of the plumulous, as well as the pennaceous parts of the breast feathers (fig. 2). No significant differences were found between the samples prior to the deprivation and those after the first bath in sand. The plumulous parts of the back feathers also showed a significantly higher level of lipids due to the deprivation, whereas their lipid level tended to be lower after the first dustbath (fig. 2). No significant deprivation or bathing effect was found for the lipid level of the pennaceous parts of the back feathers.

Within the wood-shavings treatment the level of lipids on the plumulous breast feather parts correlated to that of the plumulous back feather parts immediately after deprivation, as well as after the first dustbath ($r_S$=0.82 and 0.61; $P < 0.01$ and $P < 0.05$, respectively). The lipid level of the plumulous breast feather parts correlated to that of the pennaceous breast feather parts for both sampling periods, too ($r_S$= 0.90 and 0.74; $P < 0.001$ and $P < 0.01$, respectively). Within the sand treatment the lipid level of the plumulous breast feather parts correlated to that of the plumulous back feather parts before deprivation, as well as after the first dustbath following deprivation ($r_S$=0.63 and 0.84; $P < 0.05$ and $P < 0.001$, respectively). Further, no significant correlations per sampling period were found between the lipid levels of the different feather parts within the regions of the plumage, as well as between the regions of the plumage within the feather parts.

In wood-shavings, as well as in sand, and for all types of feather parts the amount of lipids before deprivation, the amount of lipids after the first dustbath and the change in lipid amount after compared to before the first dustbath did not correlate significantly to the amount of tossing behaviour nor to the amount of rubbing behaviour. The only exception was a positive correlation between the lipid level of the plumulous parts of the back feathers after the first bath and the amount of tossing behaviour in wood-shavings ($r_S$= 0.61; $P < 0.05$). The lipid level immediately after deprivation and the change in lipid amount due to the deprivation did show significant, positive correlations: in wood-shavings only to the amount of tossing behaviour and in sand only to the amount of rubbing behaviour. The total time spent tossing in wood-shavings correlated to the amount of lipids on the plumulous parts of the back feathers immediately after deprivation, as well as to their quantitative change in lipids due to the deprivation ($r_S$= 0.63 and 0.61; $P < 0.05$ in both cases). The time spent tossing in wood-shavings also correlated to the change in lipids on the pennaceous breast feather parts due to the deprivation ($r_S$= 0.64; $P < 0.05$). In sand the total time spent rubbing positively related or tended to relate positively to the lipid level of the plumulous parts of the breast feathers, to the lipid level of the pennaceous parts of the breast feathers, and to the lipid level of the pennaceous parts of the back feathers all sampled immediately after deprivation ($r_S$= 0.62, 0.56 and 0.54; $P < 0.05$, $P = 0.07$ and $P = 0.08$, respectively). The total time spent rubbing also positively correlated to the quantitative change in lipids on the pennaceous parts of the breast feathers due to the deprivation ($r_S$= 0.80; $P < 0.01$).

## DISCUSSION

After a deprivation of 7 to 8 days, hens took longer baths in wood-shavings than in sand. This was due to an extension of the bath only after the start of rubbing behaviour (table I) which suggests that the feed back during rubbing behaviour is crucial for the bathing programme. However, it did not imply that most rubbing behaviour was performed in the wood-shavings baths, because there was no difference in the total number of side lying and side rubbing elements between the wood-shavings and the sand baths (fig. 1). Moreover, the duration of the rubbing events, in particular the duration of the side lie element and the number of the side rubbing elements per minute of phase 2 was less for the baths in wood-shavings (table I). Especially during rubbing the litter is in intensive contact with the proximal integument when the feathers and wings are held tightly to the body. A reduced tendency to rub in wood-shavings could therefore be related to the lack of particles between the proximal parts of the integument. In contrast, tossing behaviour was not affected by the quality of the litters, because the amount of tossings and the duration of the bathing phase before the first rubbing did not differ between the substrate' treatments (table I). Moreover, the tendency to toss (vertical wing shake) per time unit of the phase 2 residue did not differ between the substrate treatments (table I). The total number of tossing events and the total number of tossing elements, however, were higher in wood-shavings than in sand (table I; fig. 1). This implies that tossing behaviour, following rubbing behaviour, occurred more frequently in wood-shavings than in sand. Although these features have to be verified for other types of litter, it is concluded that the litter quality primarily affects the facilitation of rubbing and as a consequence the inhibition of tossing behaviour. A dustbath may presumed to be a chain of functionally and causally different tossing and rubbing events which may shed new light on work on dust deprivation effects. The performance of several tossing elements and of side rubbing is enhanced as a consequence of dust deprivation (in quail: Borchelt 1975; verified in our lab with hens), but the effect on their mutual contingencies is not known. In sham-dustbaths, which develop during deprivation, a shift in the patterning of tossing and rubbing is thought to occur (Chapter 4). In contrast, Vestergaard et al. (1990) concluded that the organization developed normally for hens kept in a dustless environment. However, they did find that these hens took longer sham-dustbaths, while the frequency of side rubbing was lower compared to hens kept on sand. In scope of the previous presumption, these findings credit a review, as there might have been a significant effect on the alteration between rubbing and tossing as well.

The functional difference between the wood-shavings and the sand baths corresponded clearly to the exact location of the litter during its close integumental contact, as provided by rubbing behaviour. Wood-shavings did not reach the proximal plumulous parts of the integument, while lipids from these parts could not be removed. In contrast, sand easily reached between the feathers and excessive lipids from the plumulous parts were removed, indeed (fig. 2). Moreover, a period of wood-shavings deprivation following ad lib. bathing did not affect the level of lipids on the plumulous parts, whereas a period of sand deprivation did (fig. 2). These results prompt to investigate whether other functionalities concerning the proximal integument are affected by litter quality, too. Excessive lipids on the plumulous parts may cause the plumules to stick together and alter their fluffiness (Chapter 1), while inefficiency to remove lipids may leave them to become stale (Simmons 1964). Moreover, the reduction of skin lipids, which are produced during keratinization (Lucas & Stettenheim 1972b; Ishida et al. 1973; Hodges 1974; Borchelt et al. 1979), might depend on litter quality. A pilot sampling of the back skin of hens that had been housed either on wood-shavings or on sand showed some, though non-significant difference in thickness of the epidermal lipid layer. The epidermis had been $OsO_4$ fixated and the average thickness of the lipid layer (*SD*) was 7.3 µm (2.6) and 6.0 µm (1.3), respectively (*N*= 8 per treatment). The lipid layer of the skin included fractions of the horny layer. Excess of these fractions may become irritating and its removal from the proximal integument might be an additional function of dustbathing (cf. Kruijt 1964; Dow 1988). The functional surplus by the rubbing component within a dustbath can be tested by comparing the integument after baths that included tossing only with the integument after baths that included tossing, as well as rubbing.

Bathing in wood-shavings or in sand and deprivation of these materials affected the lipid level of the pennaceous parts of the breast feathers only (fig. 2). Compared to other regions of the plumage the distal parts of the ventral feathers are most likely to be affected, if only by the contact with the litter during lying. Moreover, changes in the amount of lipids on the breast feathers may be more pronounced as a result of a high incidence of oiling behaviour (Chapter 3).

The feather lipid condition appeared to be causally involved in the amount of dustbathing, as only the lipid levels of feather parts sampled immediately after the deprivation and/or the quantitative lipid changes due to the deprivation positively correlated to the amount of subsequent tossing or rubbing behaviour. One exception concerned the level of proximal feather lipids after bathing in wood-shavings, but the same correlation was found immediately after deprivation. It can be suggested that the exception was due to the poor wood-shavings effect on proximal feather lipids.

It is intriguing that in case of sand only the amount of rubbing, whereas in case of wood-shavings only the amount of tossing was related to lipid parameters. In sand, this relation is in line with the specific stimulation of rubbing behaviour by stale lipids on the feathers (Chapter 3), as feather lipids, accumulating during deprivation, are likely to change in quality (e.g. by oxidation: Simmons 1964). Therefore, the rubbing component of the bath seems central in maintenance of the feather lipid condition: rubbing is specifically affected by changes in the condition of feather lipids and essential in efficient removal of excessive feather lipids. In wood-shavings baths, rubbing may not be sufficiently reinforced resulting in an enhancement of tossing behaviour (fig. 1, table I). The correlation between the lipid condition and the amount of tossing in wood-shavings may therefore be a spin off from the underlying causality between rubbing and tossing. The relation between the lipid condition and the amount of tossing may have been reinforced in the course of experience, as bathing in this litter has some, though marginal effect (e.g. reduction of lipids of pennaceous feather parts: fig. 2). Several factors may explain why not all lipid parameters were associated with the dustbathing parameters. Firstly, a causal relation need not be explained in terms of lipid quantity, as lipid quantity does not relate clearly to bathing behaviour in hens (Chapter 3) and in quail (Borchelt et al. 1979). Lipid quality and related peripheral factors might have interfered causally (see earlier). Secondly, the lipid level of the one feather part ambiguously related to the lipid level of the other feather part. For instance, the levels of proximal lipids of the breast and of the back feathers did not correlate in all sampling periods. Moreover, the lipid level of plumulous parts of the breast correlated to the lipid level of pennaceous parts of the breast, but the lipid levels of the back feather parts did not correlate in this way. In order to understand these relations, which at first sight seem inconsistent, observations of oiling behaviour may prove useful. Hens oil feather parts and regions of the plumage differently (cf. Ishida et al. 1973; Chapter 3) and may have quantitatively changed this behaviour during dust deprivation.

## ACKNOWLEDGEMENTS

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## Chapter 6

# THE EXPERIENCE WITH LITTER AND SUBSEQUENT SELECTION OF BATHING SUBSTRATES IN LAYING HENS

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# THE EXPERIENCE WITH LITTER AND SUBSEQUENT SELECTION OF BATHING SUBSTRATES IN LAYING HENS

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## ABSTRACT

In the first study 4 groups of 4 laying hens each, either without litter experience (n.e.), with wood-shavings experience (wsh.e.) or with sand experience (s.e.) were observed from day 1 to day 26 while they had access to peat, sand and wood-shavings simultaneously. Preceding day 1 each of the groups and hens had been without litter for 10 days. Although at day 1, the wsh.e. hens preferred bathing in wood-shavings to bathing in the other litters, they shifted between litters from day 1 until day 7, as did the n.e. hens. After this period, however, in both treatments the majority consistently selected peat. The remaining hens selected sand as a bathing material; wood-shavings were not preferred. In contrast, the majority of the s.e. hens continued to bathe in sand, while those that shifted chose peat only, but not before day 7. As the selection of wood-shavings as a bathing material was not reinforced by experience and the development of the bathing litter selection was comparable with the n.e. hens, it is concluded that wood-shavings are inadequate as a bathing material. Sand and peat however, are adequate which may relate to the fact that these litters reached the proximal parts of the integument during bathing and that rubbing as part of the bath is performed in an uninterrupted way. Feather fluffiness, the amount of lipids on proximal feather parts and the temperature of the back were measured in the second study involving 3 groups of 16 hens each, kept either on peat, sand or wood-shavings. The temperature of the back may indicate the hen's capacity to thermo-insulate her body. The differences found between the treatments of this study correspond with the ultimate preferences found in the first one.

## INTRODUCTION

In a dustbath appetitive tossing and consummatory rubbing behaviour can be distinguished. Tossing with fluffed feathers facilitates the penetration of particles and always precedes rubbing. The latter is performed with flattened feathers and intensifies the contact between the penetrated particles and the integument (cf. Borchelt 1975 (for quail); Vestergaard et al. 1990; Chapter 2 and 4). When hens use sand during a bath, particles easily reach between the feathers and onto the skin. In this case rubbing behaviour is performed in relatively long sequences which are sometimes interrupted by tossing behaviour. However, when wood-shavings are used, the particles mostly adhere to the distal feather parts, while rubbing is repeatedly interrupted by reinitiated tossing behaviour (Chapter 2 and 5). Considering these differences, it is not surprising that some substrates are preferred to others. Chickens that have had thorough experience with wood-shavings as well as with sand are reluctant to bathe when shifted from sand to wood-shavings, whereas they bathe intensively after a reverse shift. When having simultaneous access to both litters, they prefer sand to wood-shavings (Chapter 2). However, hens familiar with sand, wood-shavings and peat prefer peat to the other litters (Petherick & Duncan 1989). This raises the question if sand and wood-shavings are adequate bathing litters at all. To answer this question the strength of the preference must be investigated (Dawkins 1980). This study does so for bathing in sand and for bathing in wood-shavings.

The phenomenon of preferring a certain bathing litter not only hints at optimal quality, but also at the fact that in the animal information is stored which promotes selection. Encountered for the first time, some litters may already be more attractive for bathing than others. Subsequently, as a result of positive experiences acquired during life this "naive" preference is thought to narrow down to a consistent choice for a specific bathing litter (Vestergaard et al. 1990; Chapter 4). If this holds for wood-shavings and for sand, hens are expected to show a conservative bathing response to the familiar material and are not expected to bathe in unfamiliar ones. On the other hand, if bathing has hardly been rewarding, then hens are expected to easily change to new substrates and to develop an unambiguous preference for the most optimal one at prolonged access. The latter is expected for hens that develop bathing habits on the barren floor and have no experience with litter whatsoever (Black & Hughes 1974; Martin 1975; Bessei & Klinger 1982; Vestergaard et al. 1990), as they prefer litter to a barren floor at repeated access (Hughes 1976; Dawkins 1983; cf. Chapter 4). Therefore, the development of a bathing preference for hens that had no prior litter experience may represent a standard response to which those of sand or wood-shavings experienced hens can be compared.

Changes in plumage condition related to bathing in different types of substrates are possibly involved in the development of a bathing preference. The mere presence of particles between the proximal parts of the integument may have an impact on future use, but so may the changes in stimulation brought about by the effects on the integument. For instance, if excessive lipids are removed by litter, this may affect the condition of the down (Healy & Thomas 1973; Borchelt & Duncan 1974; Chapter 1 and 5) and in turn thermo-insulative properties (cf. Cena et al. 1986). Thermo-insulation of the body is highly functional, as large temperature fluctuations may characterize a hen's natural habitat (Collias & Collias 1967). In a preliminary second study these integumental factors were monitored for hens on peat, sand or wood-shavings in order to verify whether these features parallel the ultimate selection of bathing materials found in the main study.

## ANIMALS, MATERIAL and METHODS

### *I. The selection of bathing materials.*

#### *Housing and Experience treatments*

Three groups of sixteen beak trimmed, 18-week-old, Warren laying hens were obtained from a commercial dealer. From hatching onwards they had been kept in wire cages with food which was not accessible for dustbathing. On arrival one group of sixteen hens was housed in a pen with wooden slats at 5 cm above the concrete floor (no experience or n.e. treatment), another group in a pen with a 5 cm layer of wood-shavings (wood-shavings experience or wsh.e. treatment) and the third group in a pen with a 5 cm layer of sand (sand experience or s.e. treatment). Each pen sized 260 x 240 x 220 $cm^3$ (l x w x h) and the groups were housed in the pens for 4 weeks. After this all hens of a treatment group were wing tagged for individual identification and randomly divided into four small groups of four animals each. They were housed in 240 x 160 x 200 $cm^3$ pens with wooden slatted floors. These pens were placed in a single row and the groups were visually isolated from each other. After 8 days 3 wooden trays of 63 x 63 x 18 $cm^3$ were placed in each pen. The trays were filled with a 10 cm layer of sand, peat or wood-shavings, but they were closed with a wooden lid. The types of litter used are illustrated in fig. 1.

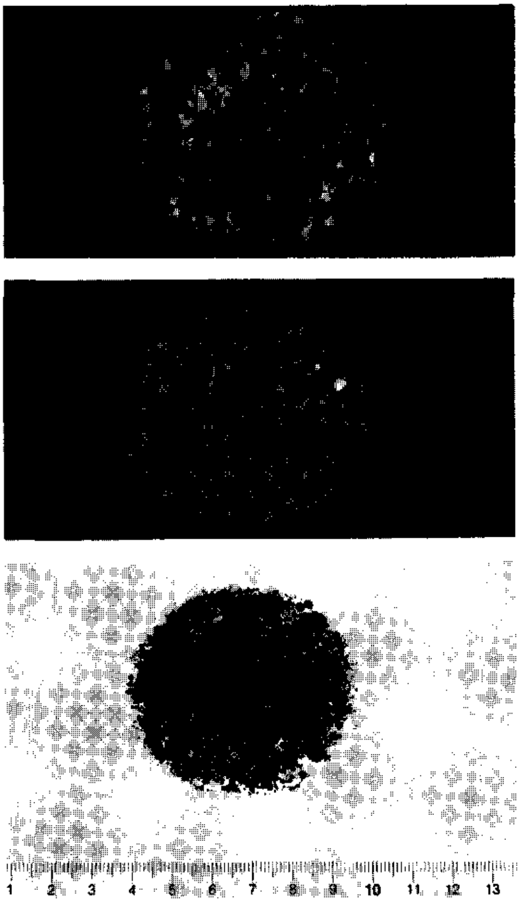

Figure 1. Litters used in this study: top: wood-shavings; middle: sand; bottom: peat. (Magnifications have been the same).

On the tenth day (hence forth called day 1) hens were given access to the different bathing litters and the observations started. A 16/8 hour light/dark cycle (lights on at 0600 hours) was maintained, while air temperature averaged 20°C and the average relative humidity was 55%. Nest boxes did not contain any litter and from a small feeding trough ad lib. food and water were provided. During the experience treatment, the n.e. hens were seen dustbathing only on the barren floor, mostly while raking the food with their bills, whereas baths in wood-shavings or sand were seen in the other treatments, respectively.

*Design and Observations*

From day 1 until day 26, each of the three trays in each of the four small pens were opened every day. During the first 11 days the trays were left open from 1300 hours until 0900 hours the next day, whereas in the following 15 days, all trays in all pens were only uncovered for one hour and a half starting at 1300 hours. Prior to opening the trays, the position of the trays was moved at random amongst the three possible locations in each pen. Once a week the trays were refilled with fresh litter.

As soon as the trays in a pen were opened, the four birds of this pen were observed for one hour and a half. The timing suited the daily distribution of dustbathing behaviour (Vestergaard 1982; Vestergaard et al. 1990). From day 1 until day 11 the hens were observed every second day. After this they were observed on days 18, 22 and 26. Dustbath behaviour and the bathing substrate chosen was recorded by means of an OS-3 microcomputer (Observational Systems Inc., Seattle, Washington). The start of a dustbath was defined as the first occurrence of vertical wing shaking; the end was marked by body/wing shaking or by other than dustbathing behaviour, if this lasted for more than 5 minutes, while shorter interruptions were considered to belong to the dustbath. A detailed description of the dustbathing elements is given in Chapter 4. Each hen's presence in the sand, peat or wood-shavings or on the barren floor was noted every two minutes in the first 45 minutes of the observation period. If a hen was seen in the nest box which occurred in less than 1 % of the observations, the hen's presence was taken to be on the floor. Eggs were never laid during the observations.

*Statistical analysis*

The number of baths and the total number of presence scores, respectively, were averaged over the four individuals in a group per type of dustbathing substrate, treatment and per observation day. Per type of dustbathing substrate these group averages were tested for the fixed effect of the experience treatment with a repeated measurements analysis of variance (model 1; SAS, 1989). Secondly, per observation day, the group averages were tested for the fixed effect of the experience treatment with a multivariate analysis of variance (model 2; SAS, 1989). In the latter case, a post hoc Duncan's multiple range test (Montgomery 1984; SAS 1989) for contrasting the responses between the experience treatments, within a dustbathing substrate was used only if the experience effects were significant.

A Spearman ranking correlation (Conover 1980) was calculated per treatment between the number of baths and the number of presence scores, each totalled per group and type of substrate over the entire observation period. The sample size was 16 (4 (groups) x 4 (types of substrates)).

### *II. The integument of hens on different litters*

Forty-eight laying hens were randomly divided into three groups of 16 birds each and housed in 260 x 240 x 220 $cm^3$ (l x w x h) pens, either with a 5 cm layer of peat or sand or wood-shavings. The hens and the lighting, food, water, temperature and humidity conditions were comparable to those in the foregoing study. After 5 months, during which all hens were seen bathing in the provided litter, the following measurements were taken within three days. On the first day, between 1300 and 1500 hours, the temperature of the surface of the plumage of the back was measured for each of the hens in the groups. A portable digital radiation thermometer (Chino IR-AH0T), which was used like a photo camera, was focussed on the plumage at the level of the hen's shoulder at a distance of about 2 metres. The temperature was recorded only when the animal was standing motionless with head held high. The final estimate of the temperature of the back was the median of a triple measurement per hen. During these measurements, the environmental temperature at hen's height was 20°C. On the second and the third day, between 1000 and 1230 hours, back feathers were sampled for subsequent extraction of lipids from the proximal parts (cf. Chapter 5). Moreover, 5 feathers from the back (7 cm caudally from the shoulder) were cut off from their base for the measurement of fluffiness. Each feather was dropped on a horizontal plane with the convex side down after which the height of the rachis carried by the plumulous barbs was measured with a micrometer at the level of the transition from plumulous to pennaceous barbs. This was repeated five times with each feather; the median was the estimate per feather, while the final estimate per hen was the median taken from the five feathers. For each group of 16 birds the following values were calculated: median amount of lipids on the proximal parts, height of the rachis carried by the down of the back feathers with the third quartile deviations (median-third quartile ranges) and average temperature of the surface of the back plumage with the standard deviation. Since this study concerned only one group per litter, no statistical comparisons were performed.

## RESULTS

### *I. The selection of bathing materials.*

In all three experience treatments, given access to the different litters, hens first showed exploratory scratching behaviour, while pecking at and sometimes swallowing the particles before any dustbathing occurred. On day 1 (table I) in the n.e. groups baths were performed on the barren floor, as well as in sand or peat. On day 1 in the wsh.e. groups 13 hens bathed in wood-shavings, but five of them shifted to peat or sand within 15 minutes after bathing in wood-shavings. The remaining three hens only bathed in peat. On day 1 in the s.e. groups all hens bathed in sand only, except one, that bathed in peat and another one, that bathed in sand, as well as on the barren floor.

Prior to day 7, the birds in the n.e., as well as the wsh.e. groups frequently changed bathing litters, while after day 7 the choice for a dustbathing litter stabilized with most hens (table I). In the n.e. groups 11 hens consistently chose peat, whereas 3 hens always chose to bathe in sand. The remaining 2 hens mostly bathed in peat, but performed a short dustbath in wood-shavings once. However, they switched back to perform a long bath in peat within one minute. In the wsh.e. groups 12 hens used peat as a bathing substrate, whereas 2 birds consistently chose sand. The remaining 2 hens mostly bathed in peat; once one of them bathed shortly in wood-shavings, but switched back to bathe long in peat within one minute; the second hen bathed shortly in sand once and did not have another bath that day.

Table I. Types of bathing substrates used either by hens that had no prior experience with a litter, hens that had prior wood-shavings experience or hens that had prior sand experience: on the first day (D1), on days 3 and 5 (D3-5) and from day 7 to day 26 (D7-26) of access to peat (P), sand (S), wood-shavings (W) and a barren floor (F); (-: no dustbaths were observed). Per treatment: 4 groups (Gr) of 4 animals each.

| NO EXPERIENCE | | | | WOOD-SHAVINGS EXPERIENCE | | | | SAND EXPERIENCE | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **Gr** | **D1** | **D3-5** | **D7-26** | **Gr** | **D1** | **D3-5** | **D7-26** | **Gr** | **D1** | **D3-5** | **D7-26** |
| 1 | F | P | P | 5 | W | S | S | 9 | S | S | S |
| | S | - | P | | W | P | P | | S | - | P |
| | S | P | P | | W | - | P | | S | - | P |
| | S | P | PW | | WP | WP | P | | S | S | S |
| 2 | S | S | P | 6 | P | - | P | 10 | S | S | S |
| | S | PS | P | | P | - | PS | | S | S | S |
| | S | P | P | | WS | SP | P | | S | S | S |
| | S | PS | P | | WP | P | P | | S | S | S |
| 3 | F | SP | P | 7 | P | S | S | 11 | S | S | S |
| | - | S | S | | W | WP | P | | S | S | P |
| | - | S | P | | W | P | PW | | P | S | S |
| | - | W | P | | W | P | P | | S | S | S |
| 4 | P | - | S | 8 | W | P | P | 12 | S | S | SP |
| | F | S | P | | W | P | P | | S | - | P |
| | S | SP | PW | | WP | - | P | | S | - | SP |
| | S | S | S | | WS | WSP | P | | SF | - | PSW |

In the s.e. group 8 hens chose sand during the entire observation period (table I). The hen that bathed in peat on day 1 bathed in sand on the following days; no hen was observed changing bathing substrates on day 3 or 5. In the period from day 7 onwards 6 animals shifted to bathing in peat only. The hen that bathed on the floor as well as in sand on day 1 showed an aberrant patterning, since from day 7 onwards, she shifted between peat and sand, while on day 26 she performed two baths in peat, two in sand as well as two in wood-shavings (table I).

Figure 2 illustrates the development of the number of dustbaths in the different litters as well as on the barren floor. In the n.e, wsh.e. and s.e. hens 65, 118 and 98 baths were observed in total, respectively: 37, 83 and 17 peat baths, 21, 15 and 77 sand baths and 4, 20 and 2 wood-shavings baths and 3, 0 and 2 barren floor baths. The experience treatment was significant for the sand, peat and wood-shavings bathing substrate (model 1: $F_{(2,9)}= 23.03$, 9.06 and 11.53; $P< 0.001$, 0.01 and 0.01, respectively). Differentiated per day the experience effects on the number of dustbaths were significant on day 1, day 11 and day 26 (fig. 2; model 2: $F_{(8,12)}= 10.76$, $F_{(4,16)}= 5.55$ and 3.11; $P< 0.001$, 0.01 and 0.05, respectively).

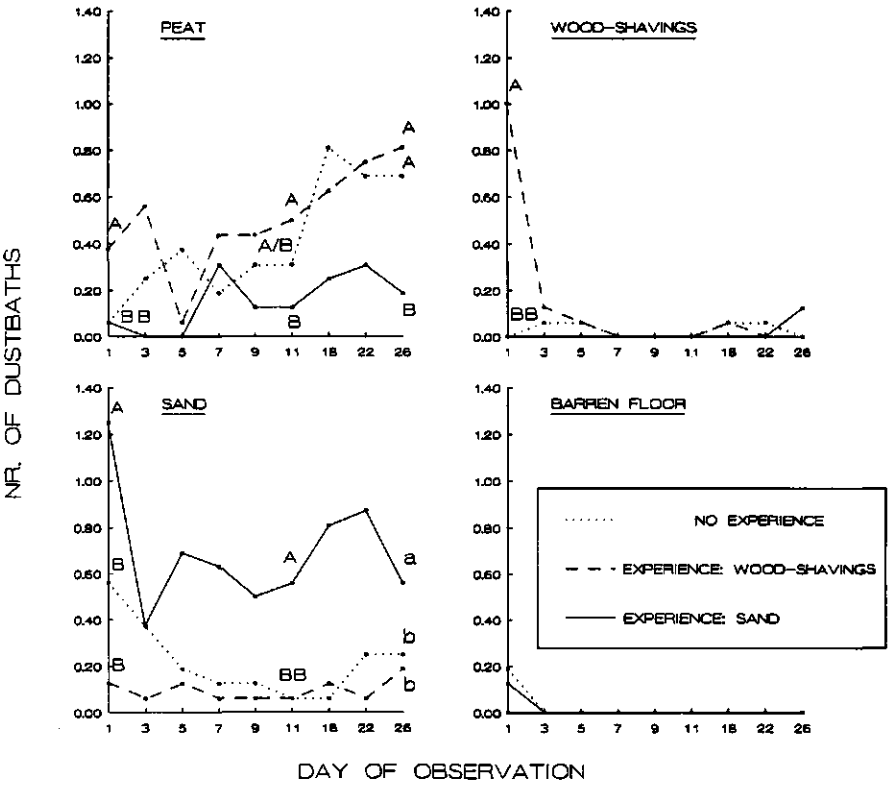

Figure 2. Average number of dustbaths per hen and day in peat, sand, wood-shavings or on the barren, wooden slatted floor during a 26 day period following no litter experience, wood-shavings experience or sand experience, respectively. Duncan's multiple range test probabilities: different lower case letters: $0.05< P< 0.10$; different upper case letters: $P< 0.05$; per treatment: $N= 4$ (groups of 4 hens each).

On day 1 the wsh.e. groups mostly bathed in wood-shavings and peat, while the s.e. group mostly bathed in sand as compared to the remaining mutually non-different other treatments (fig. 2). On day 11 and 26, however, the n.e. groups and the wsh.e. groups did not differ in their bathing response to the different litters, while the s.e. groups either responded lower (in the case of peat as a bathing substrate) or higher (in the case of sand; Duncan's multiple range test results).

On day 1 in the n.e., wsh. e. and s.e. hens, 14, 24 and 22 baths were performed, respectively and at least 80% of them were long dustbaths of 5 minutes and more which included rubbing behaviour (Chapter 2). In the observation period from day 3 to day 26, in the n.e., wsh.e. and s.e. groups, 6, 11.75 and 9.5 baths were performed each day, respectively, while in the n.e. treatment 55 % and in both other treatments about 65% of them was long. The long baths in sand and wood-shavings were in conformity with the description in the introduction. Those in peat resembled the baths in sand. Both the peat and the sand baths resulted in a visible accumulation of

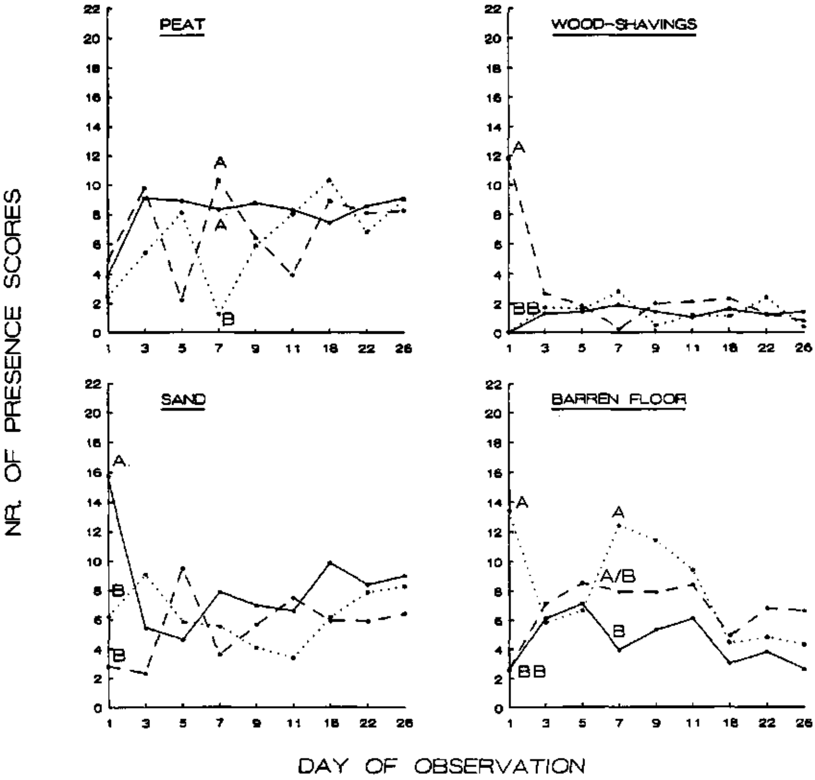

Figure 3. Average number of scores per day taken from 22 successive 2 minute interval observations, in which a hen was present on peat, sand, wood-shavings or the barren, wooden slatted floor during a 26-day period following no experience with a litter, wood-shavings experience or sand experience, respectively. Duncan's multiple range test probabilities: different capitals: $P < 0.05$; per treatment: $N = 4$ (groups of 4 hens each). Refer to fig. 2 for legend.

the material between the feathers and on the skin. In wood-shavings, however, this was hardly the case, as most particles adhered to the distal parts of the feathers. Baths on the floor lasted for about 11 min. and were all stopped after some rubbing had been initiated.

On day 1 hens were mostly present on the most familiar litter (fig. 3). In addition, the hens in the n.e. and the s.e. groups did not enter the tray with wood-shavings, but did so after day 1. After this day no clear difference in presence on the different substrates could be recognized for the three experience treatments, although for all three treatments the presence in wood-shavings seemed to remain lower than the presence in the other substrates, while the presence on the floor tended to vary much for the n.e. groups compared to the other treatment groups. The effect of the experience treatment on the total number of presence scores was significant for wood-shavings and for the floor (fig. 3; model 1: $F_{(2,9)}= 6.7$; $P< 0.05$ in both cases), but not for sand or peat ($F_{(2.9)}= 1.55$ and 1.42; $P= 0.26$ and 0.29, respectively). Per day the treatment effects were significant on day 1 and day 7 (model 2: $F_{(6,14)}= 16.75$ and 4.48; $P< 0.0001$ and 0.01, respectively; see fig. 3 for Duncan's multiple range comparisons).

In the n.e. groups, as well as the wsh.e. groups, the total number of dustbaths performed in a specific type of dustbathing substrate was not related to the total number of presence scores in that substrate in a significant way ($r_S= 0.20$ and -0.07; $P= 0.45$ and 0.78, respectively). In the s.e. groups, however, a significant positive correlation was found ($r_S= 0.72$; $P< 0.01$).

### *II. The integument of hens on different litters*

In the second study (fig. 4) the height of the rachis carried by the down of the back feathers was highest in the group that was housed on peat, intermediate in the

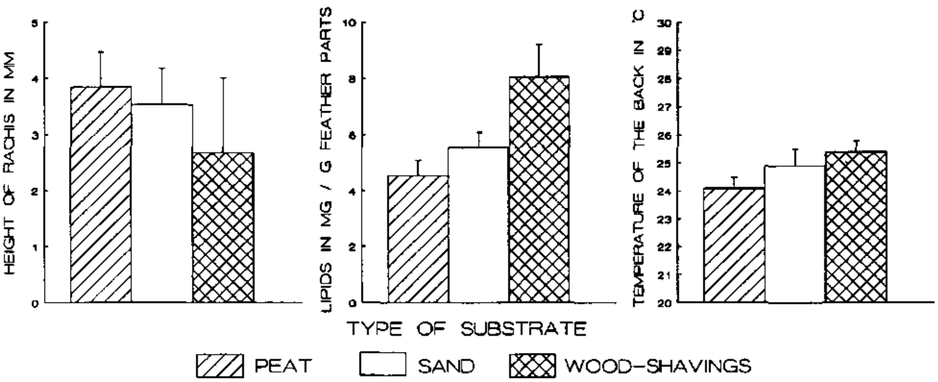

Figure 4. Median height of the rachis carried by down barbs as a measure of fluffiness, median quantity of lipids on proximal parts (with third quartile deviations) of back feathers and average temperature (with *SD*) of the surface of the back plumage in three groups of 16 hens each that were either housed on peat, sand or wood-shavings.

group on sand and lowest in the group on wood-shavings. The quantity of feather lipids on the proximal parts of the back feathers and the temperature of the back (ambient temperature 20°C) were lowest in the group that was housed on peat, intermediate in the group on sand and highest in the group on wood-shavings.

## DISCUSSION

Experience with a litter significantly affected the bathing performance. The n.e. hens clearly preferred the litters to the barren floor for bathing (fig. 2), but took less dustbaths in total and performed a lower percentage of complete long dustbaths, compared to the wsh.e. and the s.e. hens. This may relate to a lower level of familiarity in the n.e. hens with the visual and/or tactile feedback of raising particles on the feathers per se and to a lack of experience with the functional effects of litter. In the case of sand as a bathing litter, the quantity of lipids on the distal and proximal feather parts is affected, while in the case of the wood-shavings only the distal feather parts are affected (Chapter 5). Because the lipid condition of feathers in litter experienced birds relates positively to the amount of dustbathing (Chapter 3 and 5), the w.sh. and the s.e. birds may have learnt and as a result may have become more motivated, to maintain their plumage condition following a short-term litter deprivation as compared to n.e. hens (cf. Vestergaard 1980; Chapter 1 and 2). As a consequence, it was not surprising that on day 1 the majority of the s.e. and the wsh.e. hens selected their familiar litters (fig. 2). However, this appeared to be highly temporary in the case of the wsh.e. hens. Already within the first hour and a half of access (on day 1) it appeared that the wsh.e. hens did not show an unambiguous selection of wood-shavings (table I). Of all three experience treatments, the wsh.e. hens performed most peat baths, although the amount of time spent in the peat equalled that of the other treatment hens (fig. 2 and 3). Bathing in different litters occurred until day 7 and only after this day most hens consistently selected one bathing material, but they never selected wood-shavings. Moreover, it was remarkable that the change in bathing litter choices in the course of the experiment corresponded to that of the n.e. treatment (table I; fig. 2). As expected, the n.e. hens responded ambiguously to litters on day 1. In their case it was striking that, like the wsh.e. hens, these hens also "needed" about 7 days in which they experienced the different bathing materials to reach a consistent choice. Also the number of n.e. and the w.sh. hens selecting either peat or sand as a bathing medium was comparable. In both treatments the choice was independent of the presence in these litters. (It is not known why, on day 7, the n.e. groups were more often present on the floor than in the peat. However, the bathing performance (fig. 2) did not seem to be affected.) The comparable development of bathing litter selection in wsh.e. and n.e. hens shows that a hen's experience with wood-shavings hardly differs from that with a barren floor. Therefore, it is concluded that wood-shavings as used in this study (fig. 1) are inadequate for bathing.

In contrast to both other treatments, the selection of the familiar substrate was maintained in the s.e. groups. Despite the presence of another potentially attractive substrate (peat), less hens shifted between bathing materials (table I; fig. 2). Moreover, those that did shift from sand to peat, did so much later than in both other treatments producing a significant or near significant difference in the number of baths in sand and peat on day 11 and on day 26 (fig. 2). These differences cannot be explained on the basis of presence in the different litters, because after day 1 the litters were frequented at a comparable level in all treatments (except on day 7; fig. 3). It can be concluded that sand is an adequate bathing material, although for some animals it may not be optimal. Relating to this, it would be intriguing to test the reinforcing properties of access to different types of litter with operant conditioning techniques such as used by Dawkins & Beardsley (1986). Unfortunately, the litter used in their test to which access proved to be hardly reinforcing was not specified. In line with our findings it would not be surprising, if it had been wood-shavings-like.

Despite the fact that the majority of the n.e. and the wsh.e. hens chose peat (table I; fig. 2), some hens consistently used sand as a bathing litter. This supports the conclusion that sand is an adequate bathing material, optimal for some and suboptimal for other hens. The systematic differences between individuals in the appraisal of bathing material, however, are not well understood. Social restrictions or bathing space requirements did not seem to be involved, as the individuals that preferred sand were also sand bathing, while the other hens in the pen did not dustbathe. It would be interesting to verify whether these different preferences relate to other maintenance activities such as oiling or preening and to the condition of the integument (e.g. the skin keratinization, feather formation or oil gland physiology; cf. Kar 1947; Lucas & Stettenheim 1972; Abalain et al. 1984). Some flexibility in the selection of bathing substrates is maintained, however, as some individuals that preferred peat in the n.e., as well as the wsh.e. treatments "tried a bath" in wood-shavings or in sand (table I). This seems functional, as the dustbathing facilities may change in nature and the demands by the animal may change as it matures or alters physiologically e.g. during molting (cf. Dow 1988). However, the hens that consistently chose sand did not show such short "try out" baths in another particulate material. Therefore, it would be of high interest to study bathing litter selection in fowl during maturation and over a much longer period (a year to several years).

The major indicators of the adequacy of the provided bathing litter may be the penetration of the litter particles into the plumage up to the proximal integument and the performance of prolonged rubbing behaviour during a dustbath (Chapter 5). These features clearly differentiated between bathing in peat or sand on the one hand and bathing in wood-shavings on the other hand, while only the first two proved to be adequate. To explain the ultimate bathing preference additional properties of the litter, possibly related to its effects on the proximal integument must be known. In the second study (fig. 4) it was striking to find that housing on the most preferred material, i.e. peat, related to (i) most fluffiness, as measured by the height of the rachis, (ii) lowest level of the quantity of lipids on the proximal feather parts and (iii) the lowest temperature of the back, while intermediate values were found in the hens housed on sand. Down with a proper structure is thought to be a good thermo-insulator (Cena et al. 1986) and if this is indicated by the height of the rachis, this may explain the low external surface temperature of the peat hens. Hens on peat may thermo-insulate their body in a better way compared to hens on sand or wood-shavings. Some comments are in order here. Firstly, the temperature measurements may have been affected by the insulative properties of the peat, sand or wood-shavings themselves. Secondly, irrespective of the effect of dustbathing in the different litters, differences between the groups may have had some significance, for instance, a difference in social stability or in levels of feather pecking (cf. Blokhuis 1989, Vestergaard 1989). However, the differences found in fluffiness and feather lipid condition correspond to those found in other studies (Chapter 1 and 5). Therefore, they do have a predictive value with respect to the quality of bathing litters. Further research is strongly needed to specify which peripheral factors play a causal role in reinforcing the hen's use of bathing litters.

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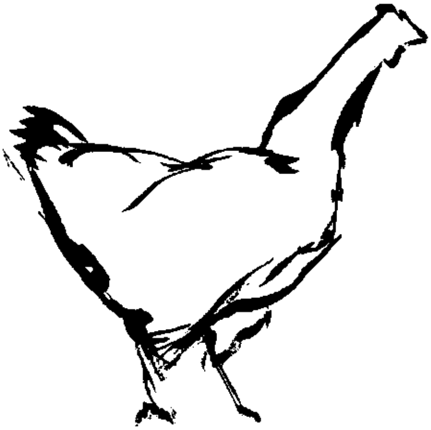

# GENERAL DISCUSSION

Feather maintenance is hard to comprehend from a human point of view. If man had a dense fur, some empathy might be possible. However, since feathers are far more complex than hairs requirements for their maintenance are bound to be more complex.

The present study has demonstrated that dustbathing in hens serves to remove lipids from the distal as well as the proximal parts of the integument. In addition, dustbathing enhances fluffiness of the feathers and this may reduce the loss of body heat. Hens show a reinforced tendency to bathe in sand and peat which are litters that penetrate the plumage onto the skin and remove lipids up to the proximal parts of the integument. Dustbaths occur as incomplete baths, that only include bill raking, head rubbing, scratching with one leg and vertical wing shaking and as complete baths, in which the afore mentioned elements are followed by side lying and side rubbing. This patterning of dustbathing behaviour strongly suggests a functional organization in which the first (called tossing) elements shape the expected condition for the second (called rubbing) ones.

Both functionality and organization of the bath depend on the nature of the litter provided. When litter such as wood-shavings is provided, particles hardly reach the proximal feather parts during bathing. As a consequence lipids are hardly removed from these parts. The rubbing behaviour is of crucial improtance, because (i) the performance with flattened feathers and wings held tightly to the body promotes the contact between litter and the proximal integument and because (ii) it is this part of the bathing chain which is markedly affected by the actual lipid condition of the plumage. The pattern of dustbathing is strongly affected by the litter quality, since in wood-shavings, as compared to sand, short, incomplete baths are more likely. Moreover, when complete baths are performed in wood-shavings, rubbing behaviour is shorter, while tossing behaviour is reinitiated frequently.

Although dustbathing in wood-shavings has no marked effect on the lipid level of the proximal parts of the feathers, it does remove lipids from the distal ones. Therefore, dustbathing in wood-shavings has some functional value. However, the hen's experience with wood-shavings has a low reinforcing value, since at the first opportunity -when given the choice to bathe in unfamiliar litters (sand or peat) or in familiar wood-shavings- the hens respond ambiguously. In fact, this response is similar to the one given by hens that, until that time, had no experience with any litter whatsoever.

Hens do develop complete dustbaths without any litter (sham-dustbathing), but these dustbaths are organized in an abnormal way after long-term deprivation. Compared to normal baths in sand, rubbing occurs sooner with these dustbaths, while the preceding quantity of tossing is reduced; sham-dustbaths may even start with rubbing or consist of rubbing behaviour only. This suggests an intrinsic reinforcement of performing sham-rubbings. Sham-dustbathing may therefore not be hedonically neutral. This is supported by the finding that hens catch up on sham-dustbathing after a temporary (3 days) prevention of this activity.

The findings in this study about functionality and organization of dustbathing suggest that bathing litters are likely to be adequate, if (i) during bathing the particles penetrate the plumage up to the skin, (ii) feather lipids are removed at the distal as well as the proximal level, (iii) rubbings are seldomly interrupted by tossings (by Warren hens lasting on average for at least one min., cf. Chapter 5) and (iv) preferences remain stable for several days at access to unfamiliar litters.

Detailed comments regarding this survey have been discussed in the preceding chapters. In the following section aspects related to the removal of integumental lipids by means of dustbathing are discussed. The second one deals with associative learning which possibly facilitates the sham-dustbath performance. The third and last section includes some general biological considerations with respect to the provision of litter in poultry farming.

## Maintenance of the integument and bathing behaviour

Striking convergent adaptations concerning bathing behaviour can be hypothesized among mammals and birds that are adapted to arid environments with large fluctuations in temperature. For instance, kangaroo rats and chinchillas inhabiting such environments have a dense insulating fur from which an excess of lipids is removed by bathing in sand (Griswold et al. 1977; Barber & Thompson 1990). The fur lipids originate from the sebaceous skin glands, but in some species extra lipids excreted by the Harderian gland are groomed onto the fur (Thiessen & Kittrell 1980). In the mongolian gerbil sandbathing and auto-grooming are thought to act in concert to optimize thermo-regulatory capabilities. This hypothesis was based on findings that low amount of fur lipids lightened the color of the fur and reduced the matting of the hair. As a consequence there was an enhanced thermal insulation of the body (Pendergrass & Thiessen 1983; Thiessen & Pendergrass 1985). In birds dustbathing is also thought to be an adaptation to arid areas (Heinroth 1955), though Galliformes inhabit dry as well as humid climates. The Red Junglefowl, the ancestor of the domestic fowl, inhabits humid tropical jungles with moderate, as well as dry and more sparsely vegetated areas at higher altitudes (up to 1200 metres above sea level) with large environmental temperature ranges (Delacour 1965; Collias & Collias 1967). Nevertheless, Galliformes only perform baths in dust (Simmons 1964) and the analogy with dustbathing in the mongolian gerbil is striking: on the one hand fowl also remove excess of integumental lipids by bathing (Chapter 1, 2 & 5), while on the other hand they enhance the amount of feather lipids by dressing the integument with uropygial gland oil (Chapter 3). Like in the gerbil's, the hen's integument becomes more fluffy as a result of dustbathing, while thermally the body seems to be insulated better (Chapter 1 & 6). Moreover, high level of lipids may darken the plumage and as a consequence may enhance heat absorption (cf. Lustick et al. 1980).

Thermal exchange depends also on the streamlining and wind proofing of the body which is realized by the pennaceous distal parts of the contour feathers (Cena et al. 1986). In natural conditions maintenance of the structure of these distal feather parts is achieved by combing-like actions of the bathing dust which is tossed and/or rubbed over/between the feathers and by combing-like actions of the bill during preening (Healy & Thomas 1973; van Liere & Romberg: to be submitted). However, hens kept in monotonous conditions may develop a way of preening (van Liere & Romberg, to be submitted) or manipulating feathers (Blokhuis 1989) which severely damages their own or their penmates' plumage. This is one of the reasons for the poultry industry to amputate part of the bill. This treatment results in a significant reduction of feather damage and of heat loss in battery caged laying hens (Herremans 1987). These features should be taken into consideration when investigating the thermo-regulatory function of dustbathing, oiling and preening in chickens.

On sunny days, dogs, cats, horses and other mammals tend to rub their body into/against dry substrates. The dust may reduce irritation, e.g. caused by an accumulation of products from the distinct sebaceous glands in the skin. Birds, too, like to bathe in dry dust (cf. Potter & Hauser 1974), possibly for the same reason, as the bird's skin can be viewed as one whole, but diffuse sebaceous gland (Lucas & Stettenheim 1972b). In addition, bathing may reduce skin irritation by sheddings of the horny layer (cf. Kruijt 1964) or by the fragments of sloughing feather shafts in moulting birds (Dow 1988). In the case of hens a rise in the environmental temperature at constant air humidity corresponds to an increase in the tendency to dustbathe (Klinger 1985). This may be caused by an increase in the irritation of the skin. Moreover, the level of stale lipids on the integument may increase when the temperature rises. This in turn may facilitate bathing behaviour (Chapter 3).

It is not known whether stale feather lipids differ from fresh ones in their impact on feather structure (cf. Chapter 1 & 3). However, the quality of the lipids on the integument may affect parasite attraction. Several Diptera (Warnes 1990; Roessler 1961; Emmens 1983) and Trematode cercariae (Zibulewsky et al. 1982; Salafsky et al. 1984; Haas et al. 1987; Feiler & Haas, 1988) sense and move towards hydrophobic metabolites produced by the skin of the host or by bacteria present on its skin. Accordingly, both the skin and the plumage of chickens contain hydrophobic substances (diesters of fatty acids) which are attractive to *Dermanyssus gallinae*, the red poultry mite (Zeman 1988). Therefore, it would be of much value to find out whether a change in lipids on the integument, e.g. due to postponement of dustbathing, lack of proper litter or to temperature changes influences this attraction. In this respect it is worthwhile to mention that non-commercial chicken breeders have found out that louse infestations are more severe in birds kept on wood-shavings than in birds kept on sand (personal communications). If it is correct to assume that stale lipids are attractive to these parasites, the difference in louse infestation between both litters may be explained by differences in bathing efficiency to remove excessive feather lipids (Chapter 5). If so, this provides an alternative functional explanation for the enhanced dustbathing response hens show to stale feather lipids (Chapter 3). Moreover, the suggested impact of dustbathing on ectoparasites (Heinroth 1955; Simmons 1964) has to be differentiated in the sense that, besides eliminating them, dustbathing may also have a preventive function as a thwarter of parasite host finding.

Interestingly, isolated cholesterol which is one of the many components of the duck preen gland, strongly attracts *Trichobilharzia ocellata* cercaria, whereas the excretion of this gland itself does so poorly (Feiler & Haas 1988). This suggests that the complex composition of the integumental excretions is well balanced in a functional sense. Therefore, it is of much interest to investigate whether birds directly monitor lipid quality of their integument (cf. Chapter 3) and, if so, what senses are involved. Birds may be specifically sensitive to the odours of the lipids on the integument and even taste or smell them, despite the assumption that their general olfactory abilities are poor[1].

The tentative nature of these aspects of feather lipid maintenance by means of dustbathing is intended to stress the limited knowledge in this respect. It is extra intriguing and complex in view of the wide range of maintenance strategies in birds. Hens, for instance, also show sunbathing behaviour which is closely associated with dustbathing in form, time and location (Huber 1989). However, no clear function of this kind of sunbathing, nor of its relation to dustbathing is known: Hou (cited in Elder 1954), suggested that ultraviolet light promotes the level of vitamin D in the oil of chicken feathers, but in other studies this could not be confirmed (Elder 1954). In addition, water birds only clean their feathers and restore their structure in water baths (cf. van Rhijn 1977b), while land species may bathe in water as well as in dust. Several land birds also allow hoards of ants to penetrate into their plumage during "baths" in ant nests (Heinroth 1955; Potter & Hauser 1974). This indicates that even within birds, maintenance strategies can diverge considerably. Caution in assuming similarities between mammals and birds is appropriate: further research into the functional aspects of dustbathing and its role in the network of maintenance activities is therefore strongly recommended.

## Sham-activities: auto-shaped Pavlovian phenomena?

There are several kinds of behaviour, that have in common that they occur in the absence of an appropriate consummatory stimulation. Sham-activities and stereotypies share this characteristic, as well as operants, that develop in the course of auto-shaping processes (Brown & Jenkins 1968, Moore 1973). In this section the biological significance of the commonality between these groups of behaviour will be discussed; in particular, the relationship between the development of sham-dustbathing (Chapter 4) and auto-shaped behaviour. This discussion seems most fruitful in motivational terms, because reinforcers will be compared (Herrnstein 1977; Hogan & Roper 1978). However, the meaning of motivation is ambiguous (Toates & Jensen 1990) and this notion in relation with the acquisition of information will be dealt with first. Subsequently, the possibility of a non-consummatory contextual stimulation initiating sham-dustbathing and the maintenance of the sham-performance

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[1] Olfactory stimuli may also mediate social behaviour: this is of particular interest in kangaroo rats, which deposit lipid signals in the dustbathing sites (Randall 1991).

will be discussed. As intrinsic reinforcement of behaviour may play an important role, its function and means are discussed next. The parallel as well as the difference between the auto-shaping process and the development of sham-dustbathing will be explained. This will culminate in a discussion of the consequences of a total lack of extrinsic reinforcement. After dealing with the possibility of a phylogenetically predisposed facilitation of sham-activities, their idiosyncratic development will be proposed.

### *Motivation and learning*

The performance of a behavioural pattern can be seen as the noticeable output of an activated neural configuration. Activation of this configuration is essential for this output, but the configuration can be activated without such an output when it is blocked by another activated configuration. Metaphorically the activation can be compared to the load of an electrical circuit: it is called the motivation of the behavioural system. This motivation is modulated by phylogenetically predisposing factors or by ontogenetically acquired ones (cf. Lorenz 1981). The first are classically called motivational factors (referring to internal causal factors, such as plasma levels of hormones, glucose etc.) or releasing factors (referring to stimuli which are aversive or attractive without experience being necessary). The ontogenetically shaped causal factors (referring to stimuli which have become aversive or attractive in the course of experience) are called conditional factors (cf. Vossen & Kop 1990). Whether or not there are good reasons to classify causal factors in motivational, releasing or conditional ones lies outside the scope of this study: its use will be maintained for the sake of simplicity. For the reasoning below it is crucial to assume that all positive, mostly functional effects of a behaviour correspond to a reduction of its motivation in the short term, while learning about the positive effects of a behaviour performed in a specific context results in an increase of the motivation when in the long term this context is encountered again.

### *Conditional initiation of sham-dustbathing*

Sham-activities which were originally termed vacuum activities (Lorenz 1981) are patterns, that "go off" without any noticeable external stimulation. It was thought that "the drive has become so strong that its motor responses break through even in the absence of a releasing stimulus" (Tinbergen 1951). The shift in terms stems from the criticism that the activation of the behaviour need not be *in vacuo*, as a weak stimulation may yet be present. Indeed, sham-dustbathing in particular, by hens kept in wire cages, is known to be facilitated and directed by the presence of food particles (Martin 1975; Klinger 1985; Vestergaard et al. 1990). However, during the dust deprivation period as described in Chapter 4, sham-dustbathing was performed on the solid wooden bottom of the dust box only and never on the wooden slatted part close to the food. The box had contained sand before dust deprivation and therefore the context of the dust box might have been conditional to positive bathing effects and have enhanced the motivation to (sham-)dustbathe. In fact it reflects the compound nature of the (un)conditioned stimulus (cf. Vossen & Kop 1990). Therefore, in addition to the classical view about sham-activities, it is suggested that sham-dustbathing as described in Chapter 4 was Pavlovian conditioned. Against this, one might reason that the solid floor of the original dust box of the cage is more attractive than the wooden slatted one close to the food, regardless of any conditioning. Indeed, Vestergaard (1981) indicated that hens kept in a dustless cage of wire with a partial solid floor (of wood), preferably sham-dustbathed on the latter. However, if one assumes that such a preference is due to differences in physical restrain on the bathing movements by these floors, this argument is not in conflict with, but supports the idea of a conditional reinforcement (see below in the subsection *Intrinsic reinforcement: function and means*). To test the hypothesis it would be interesting to accustom hens to a dustbath in close proximity of a conspicuous object and, subsequently, move a subgroup of these hens to a new, but dustless environment with that object, while another subgroup is moved to an environment without this object. The expectation is that the hens in the first subgroup develop sham-dustbathing behaviour sooner than those in the latter subgroup.

### *Intrinsic maintenance of sham-dustbathing*

If a specific context facilitates sham-dustbathing during dust deprivation, the question is if there is a reward following sham-dustbathing to maintain this contextual dependence. An intrinsic effect as a reward for sham-dustbathing seems likely as indicated by the findings of Chapter 4: (i) hens catch up on sham-dustbathing after they have temporarily (3 days) been prevented from this activity and (ii) the patterning of the sham-dustbath changes in the course of deprivation. The latter point is hypothetically represented in fig. 1 which for the sake of simplicity includes only one appetitive and one consummatory phase.

In ad lib. sand conditions, a low motivational level to bathe may only activate appetitive tossing behaviour: i.e. short incomplete baths are performed (fig. 1(A), L1; cf. Chapter 2). At a higher motivational level, some consummatory action (rubbing) may be included which will be proportional to an additional amount of appetitive preparation, as illustrated arbitrarily with L2-L4 (cf. for quail: Borchelt et al. 1973; Borchelt 1975; confirmed in our lab with hens). Without the rewarding effects of sand, however, the development of the two may be disproportional. The data of the first study in Chapter 4 show that at low motivational levels to sham-dustbathe, only appetitive tossings were performed (fig. 1 (B), L1). However, at higher levels (L2-L4) the amount of consummatory rubbing was likely to increase, while instead the tendency to toss did not increase progressively and even decreased at high motivational levels (L4). This is striking, as it illustrates a specific progressive expression of the final act in a behavioural chain, despite the absence of the consummatory stimulation. Therefore, predominantly the performance of the consummatory rubbing behaviour seems intrinsically promoted.

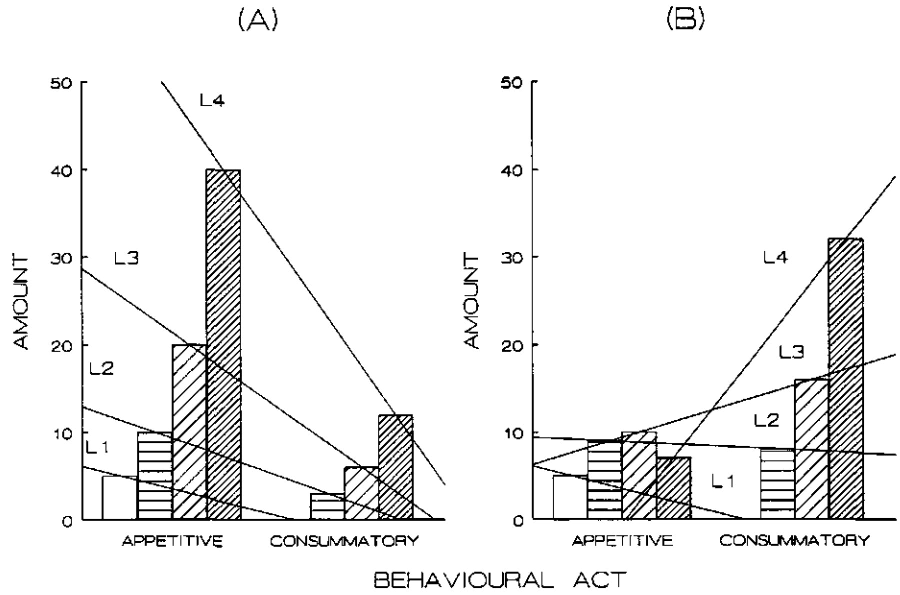

Figure 1. The hypothetical amount of appetitive and consummatory behaviour of a two-phase sequence at increasing motivational levels (L1-L4) with (A) or without (B) a proper extrinsic reward. Explanations based on dustbathing behaviour are given in the text.

## *Intrinsic reinforcement: function and means*

An intrinsic reinforcement of a behavioural programme may be functional in terms of developing and maintaining the essential sensory, neuronal and musculatury parts of that programme (cf. Toates & Jensen 1990). As a result a behaviour may be developed even before any extrinsic reward has been experienced. For instance, suckling behaviour in young mammals or pecking behaviour in young chicks may not be affected by the animal's nutritional state in the first days of life (Hinde 1970; Hogan 1989). In addition, a basic programme of social or dustbathing behaviour in chickens is presumed to develop without the appropriate consummatory context being necessary (Kruijt 1964; Vestergaard et al. 1990). Lorenz (1981) seems to support the functional significance of intrinsically maintaining the behaviour, as he suggests that a performance in accordance to the phylogenetic code is essential to learn about the most satisfactory consummatory situation. He calls this encoding within the motor pattern itself a "build-in teacher" and illustrates this with corvine birds (p. 299). These birds possess practically no phylogenetic information about what their nesting material should be like. Inexperienced birds which carry all kinds of objects to their prospective nesting places and perform -with an object held tightly in their bill-tremble shoving, do so in a fixed way regardless of the type of object. However, elongated nesting materials may get stuck as a consequence of this behaviour. This is most satisfying, because only then the tremble shoving is performed in its most violent form: not until the material really gets stuck, does the intensity of the behaviour reach a climax. After this there is a critical drop in intensity and a refractory period follows during which the bird is not interested in any kind of potential material. Chances are that the bird, while trying to tremble shove, will happen upon twigs with the above mentioned properties and, subsequently, learns to find the appropriate object for nest building. In fact, Lorenz's example neatly fits the findings in this study concerning the differences between dustbaths in different types of litter. The differences in performance predicted what litter would be preferred and would be most functional (Chapter 6). It even seems better suited to explain hens' preference for a solid floor to a wire or a slatted floor for sham-dustbathing (see the comment in subsection *Conditional initiation of sham-dustbathing*). This is based on the expectation that these substrates have hardly, if any at all, positive peripheral effect, while the solid one is physically less restraining to the sham-dustbath. An increase in positive effects of the performance per se by a decrease in physical restraint may also prove relevant to explain why hens kept in very small cages (area 847 cm$^2$) show a rebound for different kinds of stretching and shaking behaviour when transferred to larger cages (area 2310 cm$^2$; Nicol 1987).

What could be the intrinsic means sustaining a specific sham-activity? In tethered pigs oral stereotyped sham-activities performed, for instance, at a bar of the cage or a chain hung onto a bar are developed at an amount which positively correlates to the level of food restriction (Appleby & Lawrence 1987, Appleby et al. 1989). It is specifically these oral activities that are associated with endogenous opioids (at least in the first phase of development; Cronin 1985; Rushen et al. 1990). Such association seemed less clear for appetitive rooting (Rushen et al. 1990) and exploratory activities in general (Wiepkema & Schouten 1991). Similarily, food restricted broiler breeders (these are domestic chickens which have been selected to grow rapidly and thus should have a high food intake) may develop non-nutritional "spot-pecking". In turn, this may be significantly reduced in a dose-related way by nalmefene, an antagonist of the central opiate receptors (Savory 1989). Thus, an endogenous opioid reinforcement, linked to certain patterns within a behavioural sequence may form a neurobiological basis for the promotion of a specific sham-activity. In this respect, it is of interest to find out whether endogenous opioids are involved in sham-dustbathing and, if so, whether their impact differs in maintaining the appetitive or the consummatory parts.

### *Auto-shaping and sham-dustbathing: similarities*

Auto-shaping was first described by Brown & Jenkins (1968) in pigeons; at variable time intervals these birds were exposed to a key light which was turned on for 8 seconds and immediately afterwards followed by a 4-second opening of a food tray. It was crucial that both the timing of the light-food presentations and the operation of the food tray were not affected by the behaviour of the pigeons. The following stages roughly describe the afore mentioned auto-shaping process: firstly, there was a general increase in activity, particularly when the key light was turned on; secondly, the pigeons moved progressively towards the key; and thirdly, they finally performed pecking movements in the direction of the key. It is striking that during auto-shaping of hungry pigeons with light-food pairings the bill made food grasping movements towards the key, whereas in the case of light-water pairings with thirsty pigeons, it was asif the animals were actually drinking the key. This response appropriateness to the key suggests auto-shaping to be a Pavlovian phenomenon (Moore 1973). This is supported by an impairment of auto-shaping in case of contextual changes, for instance, by offering a key with a different colour during the intertrial intervals (Reilly & Schachtman 1987). When described in motivational terms, the similarity between the process of auto-shaping and the development of sham-dustbathing is as follows. In the first stage of the auto-shaping process the context is unfamiliar, the animal is aroused and general exploratory or even fleeing responses are likely to occur which may overrule their eating behaviour. In the dust deprivation experiment this stage parallels the low performance of sham-dustbathing at the start of the deprivation which is a new situation, too. In the second stage of the auto-shaping experiment a habituation is likely to have taken place and the pigeons learn to relate the light to the subsequent availability of food. As a consequence their eating motivation will be increased by the onset of the light and foraging behaviour will become likely, i.e. exploration oriented at the context which predicts food. This stage corresponds to a stage in the dust deprivation experiment where the hens have become familiar with the new dustless environment. As suggested earlier, it is likely that the hens had learnt to relate the dust box context to the positive effects conditional to dustbathing behaviour. Therefore appetitive sham-dustbathing patterns (tossings) can be facilitated here. In the third stage of the auto-shaping experiment the prediction has become reliable and the motivation to eat, which is induced by the key light, reaches an asymptotic level. Not the appetitive orientation towards the key which in the earlier stage preceded the food delivery, but the motivation-specific consummatory pecking at the key is reinforced. The performance of a sham-dustbath may have its own reward (cf. the three preceding subsections) and the dust deprived hens may learn about this consequence of their behaviour. This behaviour is located at the empty dust box and as the prediction about the consequences of sham-dustbathing in the empty dust box becomes reliable, the empty dust box will strengthen dustbath motivation. As in the auto-shaping experiment, it is not the appetitive behaviour (tossing), but the consummatory behaviour (rubbing in the dust box) which is reinforced specifically. In conformity with the interpretation of the results schematically illustrated in fig. 1B, Brown & Jenkins (1968) and Herrnstein (1977) suggested that the setting dependent development of an auto-shaped response would indicate a self-reinforcement. It is striking that the means of reinforcement in auto-shaping corresponds to the ones in sham-activities. For instance, in hungry rats that were auto-shaped to touch an extended food signalling lever the administration of a ß-endorphin antagonist (naloxone) impaired the amount of nose-pokes at the retracted lever during the intertrial intervals (Messing et al. 1989). Unfortunately, no descriptions were given of the oral activities during contact with the retracted lever and as a consequence it is not clear what act was actually reinforced. Nevertheless, also similar to the example of the oral stereotypies in tethered pigs (subsection *Intrinsic reinforcement: function and means*) the locomotor activity which was suggested to be exploratory, was not affected by the naloxone treatment. In conclusion, both in the dust deprivation experiment and in the auto-shaping experiment animals become increasingly motivated in a specific context which ultimately conditions the performance of consummatory behaviour without the actual consummatory stimulation being present. Intrinsic reinforcement seems crucial in these conditioning processes, therefore both types of development are presumed to be motivationally similar at the conditional causal level (see the subsection *Motivation and learning*)[1]. The presumed similarity between both phenomena can be tested in sand deprived hens by exposing them, at variable time intervals, to the bathing material, while they are unable to control the sand presentations. If the development of sham-dustbathing is essentially an auto-shaping phenomenon, the hens are expected to sham-dustbathe more and start to sham-rub sooner during the later intervals of sand deprivation, as compared to the earlier ones. Another way of testing the suggested similarity is to monitor how an auto-shaped response in a "trained" animal develops. The animal is kept in its "training" cage all the time with the key lighted according to the familiar scheme, but without any form of extrinsic reward for a prolonged period. It is evident that a deprivation of food or water would not be appropriate, but, for instance, the deprivation of non-nutritional chewing material which is consummatory in the Mongolian gerbil (Glickman & Schiff 1967) or of a sexual partner in the case of the pigeons (Moore 1973) would be. After a drop in the learned response, following initial deprivation (called extinction: cf. Hogan & Roper 1978), the animals are expected to become familiar with the deprived situation. They are expected to develop a motivation-specific sham-response which is similar to the auto-shaped response and more stronger related to the key than in the case of naive control animals.

### *Auto-shaping and sham-dustbathing: differences*

Looking at the development of a sham-activity and the auto-shaping of a response there is one clear difference: in the latter an extrinsic reward (food, water)

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[1] A hedonic value of auto-shaped behaviour is suggested by its cognitive characterization as superstitious (Brown & Jenkins 1968) or fetish-like behaviour (Moore 1973). At this level a similarity with sham-dustbathing can also be assumed. This idea is based on my impression of hallucination in sham-dustbathing hens, while later I read comparable ideas about sham-performances in general (Lorenz 1981). If these ideas are correct one might expect that sham-dustbathing hens are less easily disturbed than those that are sand bathing.

is eventually additional to an intrinsic one, whereas in the former the animal is deprived of the extrinsic reward. At this point, it is necessary to differentiate between the quality of these behavioural effects. In view of how motivation is defined (subsection *Motivation and learning*), all positive effects result in (i) a decrease in the motivation in the short term, next to (ii) a contextually dependent increase in the longer term. However, the effects are likely to differ qualitatively and may not be interchangeable. Referring to the example of pecking behaviour in the auto-shaped pigeon, it is presumed that pecking to the key (without any immediate nutritional consequences) has an intrinsical value (the maintenance of the behavioural programme) and may reduce the motivation. In case of pecking behaviour which is paired immediately with the uptake of food, motivational causal factors relating to the nutritional state of the animal (see also subsection *Motivation and learning*) will reduce yet another part of the motivation (this is a simplified adaptation of the phenomenon: cf. Wiepkema 1971). In fact, the concept of heterogenous summation at the causal plane of the behaviour (cf. Baerends & Kruijt 1973) is reflected at the plane of the behavioural effect. A heterogenous summation in motivation reducing effects might be assumed: the performance reduces the motivation in the aspect of behavioural maintenance per se, while the appropriate (consummatory) context may do so for other maintenance aspects. This view aligns with, for instance, the finding that domestic fowl still show nest building behaviour, even though a complete nest was presented (Hughes et al. 1989). Since a complete nest is the final effect of this behaviour, it may reduce the motivation at the releasing causal level. However, the presentation of a complete nest may not have affected the motivation at the performance level of the nest building behaviour.

In the longer term an increase in the motivation can be expected as a consequence of context dependent learning about the positive effects. If the animal is familiar with a context which predicts an intrinsic reward and an extrinsic one (e.g. food or water) conditional to a specific performance, this response will increase to an asymptotic level at repeated perception of this context (cf. Hogan & Roper 1978). This may be explained by assuming that at this level there is a balance in contextual factors enhancing the motivation and intrinsic factors, which are related with the performance and physiological maintenance, decreasing the motivation. However, if the animal is familiar with a context predicting an intrinsic reward conditional to its performance only, the behavioural performance may reach an abnormally high level. This may be explained by suggesting that the motivation will never significantly decrease, since part of it (e.g. related to glucose uptake) cannot be affected by performing per se. As a consequence, the animal maintains a high motivation and is very likely to show the behaviour as soon as it perceives the context. The context proves to be valuable by intrisic reward which further increases the motivation of the animal in a future encounter. A Pavlovian trapping may proceed, while the sham-activity culminates to the pattern which is most rewarding intrinsically. Other workers (Hughes & Duncan 1988; Toates & Jensen 1990) have put much emphasis on the role of intrinsic reinforcement in the case of increasing occurrence of sham-activities when the appropriate consummatory stimulation is absent, but here the significance of the context is emphasized, too. In comparison to functional behaviour, two effects can be expected from the suggested type of Pavlovian trapping. Firstly, it is expected that in a varied environment sham-activities only develop slowly, as the chance of perceiving the context in which sham-behaviour may be initiated is lower. Conversely, it can be expected that sham-activities become persistent in a monotonous setting, where the chances of perceiving the valuable context are high. The latter is illustrated by the oral stereotypies in the tethered sows which may persist when the sows are untethered and are given the opportunity to direct their behaviour at litter instead of the cage bar (Schouten, personal communications). Moreover, mallard drakes caged in isolation attacked their own tails and persisted in doing so even after having been released from captivity (Schutz 1965 cited by Lorenz 1981). More dramatically, a ritualization of self-punitive behaviours in restrained monkeys (Moore 1973) or injuring behaviours among farm animals kept in high densities in barren cages (Wiepkema 1987) may be induced by this mechanism. Secondly, the effect of the suggested Pavlovian trapping is that the performance level of a behaviour divorced from any consummatory situation becomes higher than the performance level of the behaviour in the functional condition. In battery caged hens without litter the daily total amount of time spent dustbathing per hen is more than four times higher than in aviary hens kept on wood-shavings (Botermans, unpublished data; cf. Rommers et al. 1991). This is similar to the long-term dust deprivation effect in Chapter 4, as the deprived test hens showed higher dustbathing levels than the control hens (at least the beak-trimmed ones). Compared to sows which were provided straw as a nesting material, tethered sows on a barren concrete floor performed such a high level of rooting behaviour during nesting that one tethered sow even injured her nose disc (Baxter 1982). In horses kept singly in a small stable without hay biting on the rim of the empty feeding trough, while sucking air, may develop notoriously fast (in one night) to levels, at which so much air is swallowed that the horse's life is in danger (Kiley-Worthington 1983, Schilder 1988, Schilder, personal communications). Impoverished housing conditions also induce extremely high levels of running in Mongolian gerbils (to the equivalent of 12-15 miles a night) compared to seminatural confinements (cf. Hogan & Roper 1978). Some tethered pigs may perform the earlier mentioned oral stereotypies for 10 hours a day (Cronin 1985). Food restricted broiler breeders show a very high level of non-nutritional spot-pecking compared to ad lib. fed ones (Savory 1989). In conclusion, it is suggested that, at the motivational/releasing causal level (see the description in subsection *Motivation and learning*), there is a significant difference between the development of a sham-behaviour deprived of its extrinsic reward and the development of an auto-shaped behaviour.

### *Phylogenetic and idiosyncratic predisposition of sham-activities*

Although sham-dustbathing as described in Chapter 4 is likely to have been facilitated in a context which had become attractive, because it previously contained sand, animals may also have phylogenetically determined (naive) contextual preferences. Hens that never had any experience with litter sham-dustbathe at locations which enable them to bill-rake in some way or another. Bill-raking occurs in food or through the plumage of a pen mate (Martin 1975; Vestergaard et al. 1990). These may be highly attractive raking locations by phylogenetic standards which seems especially true for particulate food. This assumption is based on the behaviour of quail where the normal dustbathing development is enhanced strikingly in chicks that bill raked *into* dust as compared to those that raked *at* the dust only (Borchelt & Overmann 1975). In the first weeks of their life veal calves that from birth onward are reared without their mother develop sham-suckling behaviour. At the onset of this development all kinds of protrusive body parts of pen mates are suckled. This already indicates a roughly outlined predisposed preference. At this time there is not yet a reliable relationship with the context. However, those calves that happen upon the prepuce of the pen mate will dramatically increase and maintain their preputial sham-suckling (de Wilt 1985). The ones that do not, will mostly develop a lower amount of sham-suckling on another protrusive body part. Especially the preputial sham-suckling is a type of oral stimulation and posture very similar to suckling the mother's teat; therefore, the initiation of this sham-activity could be a phylogenetically predetermined one.

However, it is significant that not all hens sham-dustbathed only, while bill raking through the food (Martin 1975; Vestergaard et al. 1990), that not all veal calves sham-suckled on the prepuce of a pen mate (de Wilt 1985) and that not all tethered pigs performed oral stereotypies to the cage bar in front of them (Schouten, personal communication; cf. Wiepkema 1987). It illustrates that even within monotonous cages the causal (conditional) development of sham-activities strongly depends on the individual's history. In fact, the individual's genetic background as well as its life history should be taken into account (cf. Baerends 1976, Hogan & Roper 1978, Toates & Jensen 1990, Wiepkema & Schouten 1991; see also the discussion in the next section). As a consequence, it may be hard to find a general stimulus pattern for different individuals performing the same sham-activity. A close examination at the individual level of exploratory activities related to an uncontrollable deprivation of the consummatory stimulus (cf. Nicol & Guilford 1991) and a careful testing of the contextual significance may elucidate how the behaviour is shaped during the deprivation period, possibly resulting in those earlier mentioned "break-throughs of vacuum activities".

## Effects related to the provision of litter in poultry husbandries.

Within most countries of the EC there is a growing public interest in banning battery cages for laying hens, but enforcement by law is likely to be postponed until aviary systems with an economically alternative value have been developed (Kuit et al. 1989). Provision of litter within the latter systems is one of the requirements to improve hens' welfare. It has been suggested that the attractiveness of the bedding for foraging (Blokhuis 1989) or for dustbathing (Vestergaard 1989) is highly relevant to reduce the occurrence of pecking at and pulling out feathers of pen mates and as a consequence to limit the development of cannibalism. Feather pecking as well as dustbathing affect quantity and quality of the feather cover of the body which in turn is essential for the maintenance of body temperature, reduction of food consumption (cf. Herremans 1987) and, thus, of faeces production. In this study other functional aspects of hens' bathing practices in litter have been suggested (e.g. prevention of host finding by parasites) which evidently do not conflict with the commercial scope and environmental constraints of poultry farming. Problems concerning the use of litter have also been mentioned: disease incidences such as coccidiosis (Braunius 1987) and the production of ammonia (Weaver & Meijerhof 1991). These are less easy to control by the farmer when litter is used. Aberrant behaviour, disease susceptibility and high energy loss are interrelated, however, raising the question whether hens are able to control their condition adequately when kept without appropriate litter.

In stress related phenomena the concept of controllability has significantly attributed to clarify and predict deviations in behavioural, physiological as well as immunological conditions (cf. Weiss 1972; Koolhaas & Bohus 1989; Levine et al. 1989; Schouten & Wiepkema 1991). Phylogenetically, animals are limited in for instance the reinforcing potential of behavioural patterns (cf. Breland & Breland 1961; Hinde & Stevenson-Hinde 1973), while within a species individuals are likely to differ genetically and to be predisposed to adopt different coping styles (cf. Duncan & Fishie 1979, Benus 1988). Therefore, (phylo)genetically individuals may be restricted in dealing with environmental variations. In addition, the experience with spatial and temporal relations acquired in life is a key item in the individual's controlling and predicting capacity. Experience with contingencies between actions and effects seems necessary to develop and maintain attentional processes as a prerequisite for future coping (Mason 1978; Wemelsfelder 1990). Moreover, the type of experience (i.e. whether a certain kind of behaviour has resulted in success or failure) may affect the type of control, for instance, the type of behavioural strategy, the activation of the (para-)sympathetic response, the fertility or the proliferation of the immune system (cf. Benus 1988; Koolhaas & Bohus 1989; Sapolsky 1990). In fact, the (phylo)genetic as well as the ontogenetic experiences bias the incentive value of the environmental stimulation or (in cognitive terms) the individual's appraisal of the environment.

Hypotheses based on the concept of controllability predominantly concerned laboratory and farm mammals, but may also be applicable to birds in general and to chickens in particular. In adopting this approach, it may be expected that in an adequate environment hens experience their behaviour to be effective and learn to control their condition in a (phylo)genetically predisposed way. They may succeed better, if the adequate environment is there from their youth up. In particular, effective interactions with litter are expected to add to the hens' learning about the control potential of their behaviour. In turn, this may prepare hens to functionally deal with unexpected circumstances (frustrations) in general. For instance, the number and outcome of social conflicts is hard to control and predict, especially when the animals are kept in an unnaturally high density and the conflicting individuals are not familiar with each other (cf. Zayan & Dantzer 1990). Indeed, experience with adequate litter does affect the appraisal of the social environment, as hens that are reared and kept without litter are pecking at penmates more and are more severe towards them than hens reared and kept on sand (Vestergaard 1989). Farmers will profit when their hens are well skilled in dealing with unexpected circumstances.

Bathing in dust may provide fowl with the appropriate consummatory stimulation, to maintain the integument. For instance, if the removal of lipids at the proximal feather parts by the bathing litter is effective, this litter may be preferred for bathing (cf. Chapter 5 & 6 and the section: Maintenance of the integument and bathing behaviour). However, fowl "specialize" in using dust, therefore hens may not have another way of achieving feather lipid maintenance (cf. the Introduction) and may remain highly motivated when deprived of dust (cf. the preceding section). The sham-dustbathing behaviour developed by deprived hens does not seem to improve the integumental condition; instead it is assumed to relate to the maintenance of the behavioural programme per se. On this basis it is concluded that not only the integumental condition, but also the dustbath motivation becomes uncontrollable when hens are chronically kept without appropriate litter. In view of how welfare is explained (cf. the Introduction) this conclusion implies that hens' welfare is reduced in the above mentioned condition. From a cost and benefit point of view a high level of sham-dustbathing (especially in a monotonous environment: cf. the previous section), specifically in hens with an active coping strategy (cf. Benus et al. 1987, Benus 1988), may result in an increased overall level of behavioural activity which in turn is not profitable for the farmer (cf. Luiting 1991). It would be of much interest to investigate whether coping behaviours, health, metabolism, growth and reproduction of hens (cf. Wiepkema 1987; Bohus et al. 1987; Sapolsky, 1990) are affected by the provision of litter and the type of litter. In view of the above this would have to be done for different strains of hens, as well as for hens that differ in (early) litter related experiences. It could be achieved by keeping different types of hens without litter or by providing them with litters of different qualities when they are growing up (from hatching) or when they are full-grown (e.g. after 18 weeks: a commercial rearing period). Subsequently, the respective effects of these treatments would be elucidated by testing the quality of the social interactions, coping in the non-social context, stress physiology, fertility, metabolism and immune responses.

The biological aspects of dustbathing as presented in this study hopefully promote to discuss the significance of (phylo)genetically as well as ontogenetically stored information (cf. Lorenz 1981) in the field of animal husbandry. If applied to other biological phenomena of fowl in particular and of domestic animals in general, this may contribute towards an understanding and predicting of abnormal behavioural developments, enhanced disease susceptibilities, high energy losses etcetera. Biologically, ethically and zootechnically, this certainly would be of great importance.

# SUMMARY

In nature fowl specialize in using dust for bathing (General Introduction). Although dustbathing seems highly adaptive, laying hens may be kept without litter, in which condition sham-dustbathing is performed. Therefore, it is biologically, ethically, as well as zootechnically of much interest to investigate the effect of dustbathing behaviour in laying hens, related to its organization in different bathing conditions. This forms the basis for this study.

In ad lib. sand conditions complete dustbaths (as explained below) last about 20 minutes and on average they are performed once every two days (Chapter 1). After short-term deprivation of sand (33 days) the daily frequency as well as the duration of bathing was increased, while within 2 days the excess of feather lipids accumulated during the deprivation period, was removed. In addition, down fluffiness was enhanced.

Hens were reluctant to bathe when shifted from sand to wood-shavings, whereas they bathed intensively after a reverse shift (Chapter 2). When having simultaneous access to both litters, they strongly preferred sand to wood-shavings. In wood-shavings most baths appeared to be short (up to 5 min.) and incomplete; they included bill raking, head rubbing, scratching with one leg and vertical wing shaking only. In sand, however, the majority of the baths was long (> 5 min.) and complete. In addition to the afore mentioned elements, they included side lying and side rubbing which were always preceded by the afore mentioned elements. Therefore, a functional organization of the dustbath, respectively based on tossing and rubbing, was presupposed. Tossing with fluffed feathers facilitated the penetration of litter particles into the plumage, while rubbing with flattened feathers and wings pressed closely to the body promoted the contact between these particles and the proximal integument in particular. After a short-term deprivation (17 days), the bathing response to wood-shavings was ambivalent as compared to sand: many short dustbaths, as well as extremely long ones were performed. Despite the reluctance to bathe in wood-shavings and the ambivalent response to wood-shavings, excess feather lipid removal was comparable to the removal after sand bathing. However, in contrast to sand, wood-shavings did not reach between the feathers and on the skin, but adhered to the distal parts of the feathers only. The nature of the litter may have affected the lipid distribution over the individual feather.

The performance after a short-term (7 to 8 days) deprivation as well as the effect on the integument was analyzed in detail for the first bath in both wood-shavings and sand (Chapter 5). The baths in wood-shavings were 30% longer than the ones in sand which was due to extension of the phase in the bath, which included rubbing. However, the rubbings were shorter than those in sand, while the tossings were frequently reinitiated. It is thought that the stimulation of rubbing depends on the rate of penetration of the litter, while in turn the reinitiation of tossing depends on the tendency to rub. Rubbing promotes the contact between the litter and the proximal integument. This corresponds to the finding that excess lipids at the proximal level of the (fluffy) feather parts was removed in sand only. The removal of

lipids at the distal pennaceous parts was achieved in both sand and wood-shavings. The quantity of feather lipids after deprivation or the change in lipids due to deprivation correlated positively with the amount of time spent rubbing in sand and spent tossing in wood-shavings. The latter relationship is thought to result primarily from a causal relation between feather lipid condition and rubbing. Rubbing is not stimulated adequately by the nature of the wood-shavings and the tendency to toss may grow subsequently.

Experience with one specific dustbathing material was studied in a choice situation, in which unfamiliar litters were offered (Chapter 6). Hens were expected to show a conservative preference for familiar litter, if this preference had been reinforced by previous experiences. If this was not the case an equivocal response was expected initially and a consistent choice was expected after a prolonged sampling of different materials; in particular such a response was expected for hens that had had no previous experience with litter at all. Although wood-shavings were preferred to a slatted barren floor and as a consequence had a certain reinforcing value, hens accustomed to wood-shavings showed an equivocal response within the first 1.5 hour of access to peat, sand and wood-shavings. Moreover, the choice patterning over time and the ultimate choice were comparable for wood-shavings experienced hens and hens with no litter experience. After having sampled the range of substrates for one week most hens preferred peat (although some developed a stable preference for sand). In peat, as in sand, particles accumulated between the feathers and on the skin, while rubbing behaviour was exhibited in a rather uninterrupted way. The hens, which had a prolonged experience with sand, maintained their preference for sand for at the least one week. In accordance with the (potential) preferences for bathing litters the level of lipids on the proximal feather parts was lowest and the fluffiness was highest for birds on peat. Both were intermediate for hens on sand and at the other ends of the scale for hens on wood-shavings. As the external surface temperature of the plumage was lowest in peat, intermediate in sand and highest in wood-shavings at 20°C ambient temperature, it may be concluded that thermal insulation of the body is highest in peat, intermediate in sand and lowest in wood-shavings. The findings on bathing functionality and organization suggest that, in contrast to sand or peat, wood-shavings as used in this study are not adequate for bathing behaviour. Litters are likely to be adequate (i) if during bathing the particles penetrate the plumage up to the skin, (ii) if feather lipids are removed at the distal as well as the proximal level, (iii) if rubbings are performed in an uninterrupted way (in Warren hens in conformity with the definition in Chapter 5, on average lasting for at least one min.) and (iv) if preferences remain stable for several days following first access to unfamiliar litters.

Body temperature maintenance in hens by dustbathing is discussed in the context of striking convergent adaptations found in dustbathing mammals (General Discussion, section: Maintenance of the integument and bathing behaviour). Besides, it is argued that host attraction of parasites (flies, trematodes, mites) by components of lipids on the feathers or skin may be prevented by lipid maintenance as part of bathing behaviour.

The effect of quantity and quality of the feather lipids on dustbathing behaviour was investigated by applying lipids to the feathers in a semi-natural way (Chapter 3). This was achieved by recording the bird's oiling behaviour. Oiling behaviour occurred twice a day on average and consisted of approximately 5 oilings. During an oiling the hen distributed with her bill oil of the uropygial gland to the feathers by means of rubbing and stroking movements, the breast receiving most. Fresh or stale uropygial gland lipids were applied to the breast by simulating oiling behaviour and subsequent dustbathing was recorded and analyzed. Only the stale lipid treatment resulted in a(n) (12%) increase in dustbath duration compared to control treatment. This was mainly due to a strong increase in rubbing behaviour. The fresh lipid treatment did not result in a clear effect. The change in quality associated with an increase in lipid content (which occurs during dust deprivation) may thus increase the motivation to bathe.

During a long-term (more than 5 months) deprivation of sand, the amount of sham-dustbathing (bathing without any litter) increased to control dustbath levels or even higher (Chapter 4). A change in quality associated with an increase in feather lipid content during sand deprivation could have been the cause. However, the motivation to dustbathe may also have been enhanced intrinsically. Early in deprivation only sham-tossing baths and later complete sham-dustbaths were performed, but in the case of long-term deprivation the sham-dustbath's rubbing occurred sooner, while the preceding amount of tossing was reduced compared to a (control) sand bath. Sham-dustbaths could even start with rubbing and consist of rubbing behaviour only, indicating a specific intrinsical reinforcement of sham-rubbing behaviour. The bathing performance was not hedonically neutral, since a temporary (3 days) prevention of the sham-dustbath performance by removing all possibilities to lie down clearly resulted in a catching up on sham-bathing afterwards. When after the more than 5 months of deprivation, sand was reintroduced in the cage no significant compensation for being deprived of sand was found, although the motivation to dustbathe had increased during deprivation. Instead, signs of conflict bathing and fear were found. These indicated that sand as a bathing material had become unfamiliar after the long-term deprivation. After some experience with sand, bathing behaviour was performed in sand only and in a normal way.

The finding that sham-dustbathing always occurred in the dust box (Chapter 4) and the assumption that sham-dustbathing is reinforced intrinsically are discussed in terms of auto-shaped Pavlovian conditioning (General Discussion, section: Sham-activities: autoshaped Pavlovian phenomena?). On the basis of past positive experiences the context is no longer arbitrary, but may be a conditional factor eliciting sham-dustbathing. When the animal has no experience at all with consummatory stimulation, the context may be attractive on the basis of phylogenetic predisposition. An intrinsic reinforcement mainly of some parts in the sham-activity chain, is thought to be responsible for a Pavlovian trapping mechanism which especially in monotonous environments results (i) in a persistence of the sham-activity within the specific context and (ii) in an abnormally high level of this activity. Such an

intrinsic reinforcement of a consummatory act, which becomes associated to a specific context without the consummatory stimulation (extrinsic reward) necessarily being present, is also assumed in the auto-shaping process. In auto-shaping and in types of sham-activity other than sham-bathing endogenous opioids are likely to form the biological basis of intrinsic reinforcement. Therefore, at the conditional level of the causation, both phenomena are suggested to be comparable. However, in auto-shaping, the level of the reinforced behaviour will not become abnormally high, as an extrinsic reward always follows the activity some time. The extrinsic reward differs from the intrinsic one, since only the extrinsic reward will reduce the motivation at the consummatory motivational/releasing causal level.

On the basis of the suggested function of dustbathing it is highly relevant to consider the quality of the bathing litter in poultry farming (General Discussion, section: Effects related to the provision of litter in poultry husbandries). Hens and farmers may also benefit from an adequate ontogenetic development of litter-related behaviour, like foraging, leading to a reduction in feather pecking and cannibalism. In general, it is suggested that an environment which, from hatching onwards, suits the (phylo)genetic predispositions, supports the learned capacity to deal with unexpected circumstances, as occurs in social conflicts. In particular, hens, which are kept without litter for a long time, are likely to develop an uncontrollable deterioration of the integumental condition and an uncontrollable tendency to dustbathe. This situation of chronic stress is expected to disbalance the hen's behavioural organization which may have important effects related to coping behaviour, development of behavioural abnormalities, stress physiology, disease vulnerability and metabolism. If a homology between the mental condition of humans and hens is accepted, it can be concluded that hens, which are kept chronically without litter, are in reduced welfare. It is of great interest to investigate the hen's adaptive capacity (cf. the factors mentioned previously) in relation to its type (strain), absence of litter, litter quality, as well as to (early) litter related experiences.

# SAMENVATTING

Baden in stof (zand, losse aarde e.d.) is verzorgingsgedrag dat van belang lijkt, omdat het bij talloze vogel- en zoogdiersoorten voorkomt. Toch is er niet veel bekend over de functie van stofbaden. In tegenstelling tot vele andere vogelsoorten baden de hoenderachtigen niet in water en lijken gespecialiseerd in het gebruik van stof. De specialisatie lijkt in een subtiele, maar uiterst variabele organisatie van de stofbadgedragelementen naar voren te komen (Introductie, fig. 1). Bij nauwkeurige waarneming valt op dat het wentelen in het substraat zich bij kippen in de basis in twee fases voltrekt. Allereerst schudt een hen met name met behulp van krabbende of optrappende bewegingen van de poten het substraat tussen de opgezette veren. Deze bewegingen kunnen meerdere malen met elkaar in samenhang herhaald worden. Hierna houdt de hen zich relatief rustig en ligt met de vleugels en veren tegen het lijf aangehouden, waarbij zij zich van tijd tot tijd middels een pootstrekking langs/tegen de ondergrond wrijft. Af en toe kunnen elementen uit de eerste fase herhaald worden, waarna die van de tweede weer volgen. Het stofbad wordt beëindigd, als de hen opstaat en het substraat uit het verenpak schudt. Een variabele organisatie van stofbadgedrag kan van belang zijn voor een functionele aanpassing aan variaties in het milieu, bijvoorbeeld wat betreft de kwaliteit van het substraat. Er kan echter een te zwaar beroep gedaan worden op de aanpassingsmogelijkheden van kippen wanneer zij langdurig van strooisel onthouden worden. Dit is zeer frequent het geval in de commerciële houderij, zoals in legbatterijen. Aangezien kippen in deze omstandigheden stofbadgedrag blijven uitvoeren, zou hun welzijn verstoord kunnen zijn. Daarnaast lijkt het voor de hand te liggen, dat adequaat stofbaden voor de stofbadgespecialiseerde hoenders een uiterst efficiënt en nuttig fenomeen is, als natuurlijke selectie hieraan vorm gegeven heeft. Dit maakt het gedrag ook vanuit een commercieel zoötechnisch perspectief interessant. Met deze achtergrond heeft het onderzoek zich geconcentreerd op de functie in relatie tot de organisatie van het stofbadgedrag in verschillende stofbadcondities.

Kippen die zand gewend waren, voerden een stofbad als hierboven beschreven gemiddeld eens in de twee dagen uit; het stofbad duurde ongeveer 20 minuten (Hfdst 1). Verstrekking van zand na ongeveer een maand onthouding leidde bij benadering tot een verdubbeling van het dagelijks aantal stofbaden en een toename van de stofbadduur. Bovendien bleek tijdens de onthouding het vetgehalte van het verenkleed toe te nemen, terwijl na opnieuw verstrekken van zand, het overtollige vet binnen twee dagen verwijderd werd. Ook bleken de donsdelen van de veren donziger te zijn na het intensieve stofbaden, volgende op de onthouding (tabel I, figuur 1; Hfdst 1). Zand, dat tussen de veren gebracht wordt en het vet opneemt, zou aldus kunnen verhelpen dat donsbaarden aan elkaar kleven. Daarbij zouden deze onderdelen van de veren door de bewegingen van het zand als het ware gekamd worden.

Vervolgens werd nagegaan of het baden in zand vergelijkbaar was met baden in houtkrullen (Hfdst 2). Kippen die bij toerbeurt zand en houtkrullen kregen (figuur 1, Hfdst 2), voerden weinig stofbaden uit in houtkrullen, als ze kort daarvoor op zand gehouden waren; in de omgekeerde volgorde vond het tegengestelde plaats (figuur 2, Hfdst 2). Bij gelijktijdig aanbieden van beide materialen had zand sterk de voorkeur, zelfs na een tijd onthouding van beide materialen (tabel II, Hfdst 2). In vergelijk met de zandconditie viel op, dat in houtkrullen veel korte stofbaden (korter dan 5 min.) uitgevoerd werden, die bovendien alleen uit substraat-inbrengende elementen bestonden. Langere stofbaden bevatten ook de lig/wrijfelementen, die alleen na de eerder genoemde uitgevoerd werden. Op basis van de vorm van de elementen en het consequente tijdspatroon van inbrengende elementen en lig/wrijfelementen (kortweg respectievelijk inschud- en zijwrijfgedrag genoemd) werd een functionele indeling van het stofbad verondersteld. Het inschudden van materiaal met opgezette veren zou ter voorbereiding dienen van het meest functionele deel van het stofbad, het zijwrijven. Het laatste gedrag zou het contact tussen het ingebrachte substraat en met name de binnenste veerdelen en de huid intensiveren. Het verschil tussen zand- en houtkrulbaden in het aantal korte onvolledige stofbaden hield aan na een 17-daagse onthouding van stofbadmateriaal. Bovendien bleken houtkrulbaden zeer lang te kunnen duren (figuur 3, Hfdst 2), hetgeen duidde op een ambivalentie van het stofbadgedrag in houtkrullen. Dit werd versterkt door de indruk, dat het zijwrijven in de lange houtkrulbaden frequent afgebroken werd, waarna de kip het inleidende inschudden herhaalde. Houtkrul leek dus niet geschikt in de vorm, die hier gebruikt werd, hoewel de totale hoeveelheid verenvet, die binnen een week na de 17-daagse onthouding verwijderd werd, vergelijkbaar was voor de zand- en houtkrulbadende hennen (tabel III, Hfdst 2). Het viel echter op, dat de houtkrullen niet tussen de veren reikten en aan de toppen van de veren haakten, terwijl het zand makkelijk tot op de huid kwam. De verdeling van de vetten over de individuele veren kon dus sterk verschillen.

In een later experiment werd de vergelijking tussen zand- en houtkrulbadende hennen in detail uitgevoerd (Hfdst 5). Het eerste bad na een week onthouding van stofbadmateriaal bleek in houtkrullen ongeveer 30 % langer te duren dan in zand. Dit bleek alleen het gevolg te zijn van een verlenging van het zijwrijfdeel van het bad (het deel vanaf het eerste zijwrijfgedrag tot het einde van het stofbad); in het ervoor uitgevoerde inschudgedrag was geen verschil aan te geven (tabel I, Hfdst 5). Er werd echter niet meer zijgewreven; in totaal feitelijk net zoveel als in het zandbad (fig. 1, Hfdst 5). De verlenging van het zijwrijfdeel was het gevolg van het frequent herhalen van inschudgedrag na relatief korte zijwrijfhandelingen. De aard van het stofbadmateriaal had dus een sterk effect op de organisatie van het gedrag, waarin de stimulatie van het zijwrijfgedrag een cruciale rol speelde. De functionaliteit van het zijwrijfgedrag, waardoor met name een intensief contact tussen het ingebrachte substraat en de binnenste veerdelen tot stand zou komen, werd onderbouwd met het gegeven, dat alleen de kippen op zand in staat waren het overtollige vet van de donzige basisdelen van de veren te verwijderen. Overtollig vet van de topdelen werd door kippen op zand, zowel als door kippen op houtkrullen verwijderd (fig. 2, Hfdst 5). Het vetgehalte direct na zandonthouding bleek positief gerelateerd aan de daarna in zand uitgevoerde hoeveelheid zijwrijfgedrag. Dit kon duiden op een oorzakelijk verband, hoewel de specifieke rol van de hoeveelheid vet op zich niet duidelijk was: de vetkwaliteit of de structuur van het dons konden ook veranderd zijn tijdens de onthouding. Het vetgehalte direct volgend op de houtkrulonthouding was positief gerelateerd aan de hoeveelheid inschudgedrag daarna. In het licht van de gewijzigde organisatie van het houtkrulbad, werd dit als een afgeleid resultaat gezien van de onderbroken neiging zij te wrijven.

Uit de literatuur bleek, dat kippen een voorkeur hebben voor turfmolm als baadmateriaal boven zand of houtkrullen. Dit bood niet alleen een goede gelegenheid eerder genoemde ideeën over functie en organisatie opnieuw te toetsen, maar ook een mogelijkheid na te gaan of een minder geliefd stofbadmateriaal minder, dan wel niet geschikt is (Hfdst 6). Voor het laatste gold een redenatie conform het speekwoord "wat een boer niet kent, dat eet hij niet". De veronderstelling was, dat een onbekend, maar potentieel zeer geschikt substraat langere tijd genegeerd zou, worden bij een gelijktijdige presentatie van bekend en geschikt substraat. Bij een gelijktijdige presentatie van bekend, maar niet geschikt materiaal zou het meer geschikte echter snel gekozen worden. Dieren die nooit strooisel van welke kwaliteit dan ook ervaren hadden bleken inderdaad nu eens in het ene, dan weer in het andere type substraat een stofbad te nemen. Bovendien bleek dit het geval voor dieren met langdurige houtkrulervaring: binnen het eerste anderhalve uur van de keuzemogelijkheid tussen zand, houtkrullen en turfmolm (fig. 1, Hfdst 6), gebruikten ze naast houtkrullen ook zand en turfmolm voor hun stofbad (tabel I, Hfdst 6). Beide groepen dieren, -die zonder strooiselervaring en die met houtkrulervaring- vertoonden een sterk overeenkomend keuzepatroon: uiteindelijk werd na 7 dagen een voor beide groepen vergelijkbare vaste keuze bereikt. Het merendeel koos voor turf, een klein aantal voor zand; houtkrullen werden nooit gekozen (tabel I, fig. 2, Hfdst 6). De ervaring met houtkrullen als stofbadmateriaal bleek dus niet veel te verschillen van een naïviteit omtrent baden in strooisel. Hiermee in tegenstelling (en conform de tevreden boer) bleef het merendeel van de zand-ervaren dieren op zijn minst gedurende een week bij de keuze voor zand. Net als het zandbad, leidde het turfmolmbad tot een ophoping van materiaal tussen de veren en op de huid; het zijwrijfgedrag werd ook nauwelijks onderbroken. Daarnaast bleek het vetgehalte van de donsdelen het laagst, de donzigheid het hoogst en de temperatuur van het verenoppervlak het laagst voor dieren op turfmolm. De waarden voor dieren op houtkrullen waren hieraan tegengesteld en ze lagen er tussenin voor dieren op zand (fig. 4, Hfdst 6). Deze gegevens correspondeerden met de voorkeursresultaten en bevestigden het belang van de eerder genoemde functie en organisatie van het stofbad voor de kip. Ze voegden eraan toe dat het type substraat mogelijk van belang is voor een reductie van het verlies aan lichaamswarmte.

Op basis van bovengenoemde resultaten wordt in de Generale Discussie voorgesteld, dat strooisel voor stofbaden adequaat is (i) als tijdens het bad de partikels tussen de veren tot op de huid komen, (ii) (veren)vet verwijderd wordt op distaal, zowel als proximaal niveau, (iii) zijwrijfgedrag langdurig uitgevoerd wordt (bij Warren hennen conform de definitie in Hfdst 5 gemiddeld minstens 1 minuut durend) en als (iv) de keuze voor dit substraat ongewijzigd blijft gedurende enige dagen keuzemogelijkheid met onbekende substraten.

Warmte-isolatie van het lichaam met behulp van stofbaden wordt besproken in samenhang met frappante convergente adaptaties van stofbadende zoogdieren (Generale Discussie, eerste sectie). Daarnaast wordt aangegeven dat bepaalde vetachtige componenten van het integument van de gastheer een rol spelen in de aantrekking van parasieten (vliegen, muggen, trematode wormachtigen, mijten). Dit aantrekken van parasieten zou voor de kip in het bijzonder beïnvloed kunnen worden door stofbadgedrag.

Zoals aangegeven leek een oorzakelijk verband te bestaan tussen de hoeveelheid verenvet na substraatonthouding en het stofbadgedrag. Echter, tijdens onthouding leek het ook waarschijnlijk dat de verenvetkwaliteit aanmerkelijk veranderde. Het vet zou, zonder van de veren verwijderd te worden, immers lang aan de lucht bloot gesteld zijn en kunnen verschalen. Welke van beide factoren -kwantiteit of kwaliteit van het verenvet- was nu van belang in de veroorzaking van stofbadgedrag? Om dit te beantwoorden werd stuitkliervet op het verenkleed aangebracht en werd geregistreerd hoe deze behandeling het stofbadgedrag beïnvloedde (Hfdst 3). Het aanbrengen van vet zou echter op een natuurlijke wijze moeten gebeuren, ter vermindering van storende bijwerkingen. Daartoe werd allereerst geobserveerd hoe de hen zelf tijdens de inoliepoetsbeurten stuitkliervet op haar verenkleed brengt (fig. 1, Hfdst 3). Dagelijks bleken hennen zich twee maal in te oliën en het betrof ongeveer 5 inoliebewegingen per keer. Tijdens een inoliebeweging werd vet bij de stuitklier op de snavel gebracht en vervolgens met de snavel door middel van halende en wrijvende bewegingen gedistribueerd over de veren. Met name de borst van het dier bleek het meest ingevet te worden (fig. 2, Hfdst 3), hetgeen mogelijk duidde op extra attentie voor de conditie van de borst. Het inoliegedrag werd nagebootst met een kunstmatige snavel, waar ofwel vers stuitkliervet, ofwel oud stuitkliervet opgebracht was. In het geval van de oudere kwaliteit was het vet langdurig aan de lucht bloot gesteld geweest. Het op de vettoediening volgende zandbad bleek eigenlijk alleen door het opgebrachte oude vet beïnvloed te worden. Het stofbad was langer dan controleniveau, waarbij met name het zijwrijfgedrag toegenomen was (tabel I, fig 3, Hfdst 3). Het opbrengen van vers stuitkliervet had geen effect op de stofbadduur; het effect op het zijwrijfgedrag was niet eenduidig. Met name de kwaliteit van het accumulerende vet op de veren leek dus een oorzaak van de neiging tot stofbaden in het algemeen en zijwrijfgedrag in het bijzonder.

Dit gegeven kon van belang zijn voor het optreden van schijnstofbadgedrag (Hfdst 4). Tijdens onthouding van stofbadmateriaal leek echter bovendien een intrinsieke bekrachtiging van dit gedrag een rol te spelen. Hennen schijnstofbaadden binnen 1 à 2 weken zandonthouding en kozen steeds de houten bodem van de bak, waar oorspronkelijk zand in gezeten had. De hoeveelheid schijnstofbaden nam toe naarmate de onthouding langer duurde (fig 1, 2, Hfdst 4), terwijl het karakter van het schijnstofbad gedurende de onthouding veranderde. Waren de eerste schijnbaden korte en onvolledige, alleen bestaande uit inschudgedrag, later werd ook het zijwrijfgedrag toegevoegd. Op zich is dit niet vreemd: er was een proportionele verhoging te verwachten van de uitvoering van alle elementen als de motivatie verhoogd werd. Echter, na lange deprivatie (meer dan 5 maanden) werd het inleidende schijninschudden in tijd en hoeveelheid gekort (tabel I, Hfdst 4), waarbij schijnbaden zelfs met het zijwrijfgedrag startten, of volledig uit zijwrijfgedrag bestonden. Het dier bleek dus een intrinsieke organisatie te kennen waarin met name het zijwrijfgedrag bekrachtigd wordt. Het vermoeden dat er een interne drijfveer bestaat voor de uitvoering van het schijngedrag bleek ondersteund te worden door het resultaat, dat hennen het schijnstofbadgedrag bleken in te halen nadat het een korte tijd (drie dagen) verhinderd was (fig. 4, Hfdst 4). Een looprek als bodem in de kooi met forse openingen tussen de stokken (fig. 3, Hfdst 4) bleek hiervoor de methodologische oplossing, omdat dit de hennen belette te gaan liggen. In tegenstelling tot de toegenomen stofbadmotivatie tijdens de zanddeprivatie, bleek het zand na de lange onthouding geen extra stimulans voor stofbadgedrag. Het stofbaden verliep zelfs geremd, of ging gepaard met vluchtgedrag; andere dieren bleven op afstand van de locatie van het nieuw ingebrachte zand schijnstofbaden. Dit wijst erop dat de hennen het zand als stofbadmateriaal niet herkenden. Pas na verloop van tijd bleken de hennen geleerd te hebben het zand normaal te gebruiken.

Het interne belang van schijnstofbadactiviteit verwijst naar het idee dat gedrag dat geen functioneel effect sorteert, op een bijzondere wijze is georganiseerd: het lijkt zichzelf te bekrachtigen. In de Generale Discussie (tweede sectie) wordt een parallel gesuggereerd tussen schijnstofbadgedrag en zelfaangeleerd gedrag (in de leerpsychologie auto-shaped behaviour genoemd). Een voorbeeld van het laatste: duiven leren zichzelf een soort eetachtig pikken naar een lampje aan, dat steeds na 8 seconden branden voer oplevert. In schijnstofbaden zowel als zelfaangeleerd gedrag wordt de eindhandeling van een bepaald gedrag (zijwrijfgedrag en eetachtig pikken) uitgevoerd zonder dat de extrinsieke bekrachtiger (respectievelijk zand en voer) noodzakelijkerwijs aanwezig is. Deze specifieke bekrachtiging wordt in beide gevallen dan ook van een (soortspecifieke) intrinsieke aard gedacht. Het lijkt waarschijnlijk dat de door het lichaam zelf geproduceerde opiaatachtige stoffen als biologische basis hiervan geduid kunnen worden. De context van handeling is niet willekeurig voor het individuele dier, maar oorzakelijk met de bekrachtiging van het gedrag verbonden. In het hier beschreven schijnstofbaden zou het oorspronkelijk zand bevattende deel van de kooi de specifieke conditionerende context kunnen leveren, zoals dat ook voor het lampje geldt bij het zelfaangeleerde pikgedrag. De oorzakelijkheid heeft een individu-eigen, ervaringspecifieke oorsprong, terwijl voor schijngedrag in het algemeen (d.w.z. die activiteiten, die zonder een duidelijke natuurlijke stimulatie zich lijken te voltrekken) de oorzakelijkheid van soorteigen oorsprong kan zijn. Het schijngedrag zou binnen de aantrekkelijke context door de intrinsieke bekrachtiging bij gemotiveerde dieren een persistent karakter kunnen krijgen, hetgeen het meest waarschijnlijk lijkt in een monotone omgeving, omdat door de geringe variatie de kans om de context waar te nemen hoog is. Bovendien is het waarschijnlijk dat dan het gedrag abnormaal vaak uitgevoerd wordt. Het laatste is niet het geval bij zelfaangeleerd gedrag, omdat in tegenstelling tot schijngedrag de extrinsieke bekrachtiger uiteindelijk wel gepresenteerd wordt. Het effect van de extrinsieke bekrachtiger -in het bovengenoemde voorbeeld: het effect van voeropname op de energiebehoefte van de duif- is niet uitwisselbaar met het intrinsiek bekrachtigende effect van de uitvoering van het eetachtig pikken op zich. Het resultaat is dat in zelfaangeleerd gedrag een daling in de motivatie bewerkstelligd wordt, die tot een beheersing van de hoeveelheid gedrag in kwestie leidt. Resumerend lijkt het schijnstofbaden zelfaangeleerd in een specifieke context op basis van intrinsieke bekrachtiging, echter zonder dat een adequate voldoening (motivatiereductie) optreedt.

In de kippenhouderij is het van belang de effecten van adequaat strooisel te beschouwen (Generale Discussie, derde sectie). Niet alleen, omdat vanuit de voorgestelde functies van stofbadgedrag aangenomen mag worden, dat energetisch te besparen valt, maar ook, omdat aan andere strooisel gerelateerde gedragingen een functionele gerichtheid gegeven wordt. Zo zijn verenpikkerij en kannibalisme ervaringsafhankelijke abnormaliteiten, die een dergelijke gerichtheid missen. Bovendien kan gedacht worden aan het meer algemene positieve effect van een adequate omgeving op de vaardigheden in onverwachte omstandigheden, zoals sociale conflicten. De genetische aanleg, zowel als de in het leven opgedane ervaring in het effectief omgaan met dergelijke omstandigheden spelen hierin een essentiële rol. Gebrek aan substraat lijkt de conditie van het verenpak te verslechteren en kippen op basis van de soorteigen constitutie in een onbeheersbare neiging tot stofbaden te brengen. Dit lijkt chronisch stressvol en kan de algemene gesteldheid in relatie tot de organisatie van het gedrag, de (stress)fysiologie, het metabolisme en immuniteit uit balans brengen. Het al of niet accepteren van een homologie met de menselijke gesteldheid, bepaalt of het uit balans zijn ook voor de mentale conditie, het welzijn van kippen, geldt. Het is van belang genoemde factoren te onderzoeken voor verschillende typen kippen (rassen), die op (verschillende typen) strooisel of zonder strooisel gehouden worden en deze te relateren aan eerder (in de vroege jeugd) opgedane substraatervaringen.

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# Curriculum vitae

Het onderzoek dat in dit proefschrift is beschreven, is de resultante van een aanstelling van medio 1987 tot medio 1991 van Diederik Walter van Liere aan de Landbouwuniversiteit te Wageningen. Diederik van Liere is in 1958 geboren in Bankstown (Australië) en heeft in Nederland het basisonderwijs en het VWO gevolgd. Van 1976 tot 1984 heeft hij Biologie gestudeerd: de kandidaatfase in Utrecht en de doctoraalfase in Groningen (bijvakken: populatiegenetica, dierfysiologie, $1^e$ graads didactiek; hoofdvak: ethologie). Tijdens de Biologiestudie heeft hij assistentschappen verricht op het gebied van de histologie, de evolutie van Vertebraten en de ethologie. Eind 1984 tot medio 1986 heeft hij onderzoek aan de functie van stofbadgedrag bij kippen gedaan, hetgeen wellicht heeft bijgedragen tot bovengenoemde aanstelling. In het tussenliggende jaar heeft hij het diploma Statistisch Analist VVS behaald.