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DUSTBATHING BEHAVIOUR OF UROPYGIAL GLAND EXTIRPATED DOMESTIC HENS

EFFECTS OF DUST DEPRIVATION

By

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NØRGAARD-NIELSEN, G. and K. VESTERGAARD: *Dustbathing behaviour of uropygial gland extirpated hens. Effects of dust deprivation.* Acta vet. scand. 1981, 22, 118—128. — Dustbathing behaviour of uropygial gland extirpated and intact White Leghorn hens was studied after 27 and 75 h of deprivation of dust. Thereafter the hens were given access to dust (litter) and latency and duration of their first dustbathing bout were recorded. A short latency and/or a long duration were taken to indicate a strong urge to perform dustbathing. Judged by either of these criteria the gland extirpated birds showed a higher dustbathing tendency than the intact birds and in both groups the dustbathing tendency increased from 27 to 75 h of deprivation. Because of these findings the present study failed to support the "lipid regulation theory": that the amount of lipid in the plumage should regulate the dustbathing tendency. Rank in the social hierarchy significantly influenced latency and duration of dustbathing but there was no simple correlation. After two weeks with constant access to litter no differences in the amount of feather lipids could be found between gland-extirpated and intact birds.

dustbathing; uropygial-gland-extirpation; dust deprivation; domestic hens.

The influence of dust deprivation on dustbathing behaviour of gallinaceous birds has been examined in a number of studies in the Bobwhite quail (*Colinus virginianus*) (*Borchelt et al.* 1973, *Borchelt* 1975), the Japanese quail (*Coturnix coturnix japonica*) (*Benson & Schein* 1965) and the domestic hen (*Vestergaard* 1981a).

In the domestic hen *Vestergaard* found a gradual increase in the tendency to perform dustbathing when depriving the hens of litter for from 5 to 105 h, and similar results have been

described for Bobwhite quails by *Borchelt et al.* and *Borchelt*. Very long lasting deprivation may further elevate the dustbathing tendency since it is well known that hens kept without access to dust for several months perform vacuum dustbathing activities (*Black & Hughes 1974, Martin 1975, Vestergaard 1981b*). However, little is known about the mechanism of the deprivation effect and of the long-term temporal regulation of dustbathing. The theory which has been discussed most thoroughly is the "lipid regulation theory" of *Borchelt* and colleagues (*Borchelt et al., Levine et al. 1974, Borchelt 1975*).

According to this theory lipids from the uropygial gland are accumulated in the plumage and dustbathing is stimulated whenever the amount of lipid increases. Thus it should be the accumulation of lipids in the plumage that causes the gradual rise in the dustbathing tendency during deprivation. The theory is supported by studies on Bobwhite quails which have shown that the amount of feather lipids increases during dust deprivation (*Borchelt & Duncan 1974*). It is also known that lipids from the uropygial gland are brought to the feathers during preening by a special oiling behaviour which is part of the feather maintenance behaviour of birds (*Simmons 1964*).

Whether or not the theory is true can be tested by deprivation studies of uropygial-gland-extirpated birds. In such birds there will be no accumulation in the plumage of lipids from the uropygial glands and as a consequence the birds should dustbathe less than intact birds and deprivation should fail to increase the dustbathing tendency.

The aim of the present investigation was to examine the validity of the "lipid regulation theory" by measuring the dustbathing performance of uropygial-gland-extirpated hens after dust deprivation for two different periods of time and comparing with intact birds. Additionally the amount of lipids in the plumage was measured in order to assess the effect of gland extirpation.

MATERIALS AND METHODS

Experimental animals

Ten White Leghorn hens, 18 weeks old, were purchased from a commercial dealer. They have been raised in a deep-litter house with about 8000 hens. The birds were individually marked by the aid of different speed marker colours and allocated to two identical pens with five birds in each. All the hens in one group

were uropygial gland extirpated ("operated") at 22 weeks of age. The hens in the other group ("controls") were handled about as much as the operated hens but no sham operation was made. The rank order was determined throughout the experiment and was found to be stable and linear in both groups. One hen became ill and was removed as was a hen from the other group before the start of the experiment.

Experimental pens

Each pen measured $2.00 \times 1.75 \times 2.80$ m. They were both placed in a room in which there were no other animals. A solid wall separated the pens so that the two groups were visually isolated from each other. The front of the pens was made of wire mesh and the other walls were solid. About three quarters of the floor area in each pen was occupied by litter consisting of straw mixed with fine dry soil and sand in equal amounts. The litter was kept constantly dry throughout the experiment.

Two wooden frames with wire mesh covered the litter during deprivation periods. A black sheet of plastic was attached beneath the frames so that the birds were both physically and visually separated from the litter. During periods with access to the litter the frames were placed along the side walls of the pens. At the rear end of each pen were two nests with straw and a dropping pit with perches. A "waiting box" in which the hens could be locked up was placed on the roof of the nests.

The hens were fed ad lib with a layers ration presented from a single feeding trough and water was available from an automatic waterer. Eight 40 W neon tubes were on from 6 a.m. to 8 p.m. and there was no additional source of light. The illumination at floor level was 340 lx. Two thermostatically controlled radiators were adjusted to maintain the temperature at at least 21°C, but fluctuations between 18 and 28°C occurred during the experiment. The relative humidity varied between 45 and 55 %.

Experimental procedure

The experimental procedure was as described by Vestergaard (1981a): Before each deprivation period the hens were locked up in the "waiting box" while the wire floors were placed above the litter. Then the birds were released. At the end of the deprivation period the birds were locked up in the "waiting box"

while the wire floors were removed. Latency was timed for each bird from its release to the occurrence of the first vertical wing-shaking. The duration was recorded for each individual from the first vertical wing-shaking until the bird had risen to its feet and performed the first body/wing-shaking. Both these patterns have been described by *Kruijt* (1964).

Each group was deprived 15 times for 27 h and 15 times for 75 h, and the deprivation periods were invariably terminated at 1 p.m. The two kinds of treatments were presented in random order, and after each deprivation period the birds had full access to litter for at least two days. The deprivation levels applied and the time of day chosen for testing were based on previous experiments with intact birds (*Vestergaard* 1981a) in which the conditions had been optimized for demonstration of the deprivation effect. Data on hens which performed both dustbathing and nesting behaviour during the test were discarded.

Lipid extraction from feathers

Two weeks after the end of the deprivation experiment the birds were killed and the lipid extracted from 10 g not visibly contaminated feathers from each hen, according to the method of *Folch et al.* (1957). Since the filter-paper used permitted penetration of some particulate material the residual was dissolved in chloroform and filtered through at 8 μ millipore filter.

Statistical analysis

The duration and latency data were statistically evaluated by an analysis of variance. Independent variables were \pm gland extirpation, deprivation level (27 or 75 h) and rank in the hierarchy. The latency values were transformed to logarithmic (ln) values in order to ascertain approximation to a normal distribution. Differences between operated and control hens in lipid content of the plumage were tested by the Mann-Whitney U-test, two-tailed (*Siegel* 1956).

RESULTS

Dustbathing behaviour

During the 240 different units of observation (eight birds deprived 15 times at two deprivation levels) 215 events of dustbathing occurred. Of these, four observations from intact birds

and five observations from operated birds were excluded from the statistical evaluations because the birds showed nesting behaviour during the test.

The analysis of variance (Table 1) showed that gland extirpation, deprivation level, and rank in the hierarchy significantly influenced both latency (ln latency) and duration of dustbathing. There were no significant interaction effects.

Table 1. Analysis of variance of the dustbathing durations and ln latencies (in seconds).

	\pm Uropygial gland (UG)	Deprivation level (DL)	Rank (R)	UG \times DL	UG \times R	DL \times R	UG \times DL \times R	Error
	Mean squares							
ln latency	7.85**	22.65***	9.02***	0.14	1.71	0.75	0.73	0.77
Duration	452×10^4 ***	793×10^4 ***	110×10^4 *	6×10^4	64×10^4	67×10^4	4×10^4	32×10^4
DF	1	1	3	1	3	3	3	190

*** $P < 0.001$ ** $P < 0.01$ * $P < 0.05$

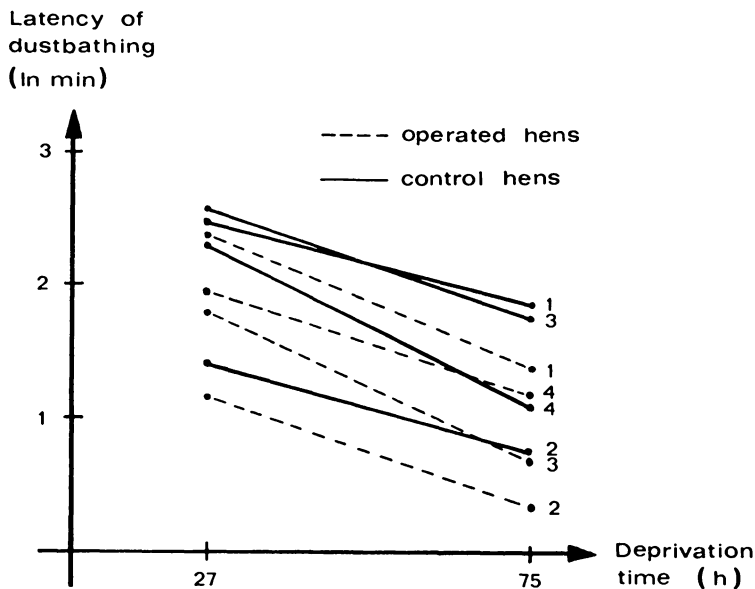


Figure 1. Latency (ln minutes) for dustbathing after 27 and 75 h of deprivation for 4 uropygial gland extirpated ("operated") and 4 intact ("control") hens. Number indicates rank in the hierarchies. Each point represents the mean of 10–15 observations.

The directions of the effects appear from Figs. 1 and 2. The ln latency (Fig. 1) decreased in all hens from 27 to 75 h of deprivation and the values of the operated hens were smaller than those of the controls. There was no linear correlation between rank and ln latency, but with one exception the mean values (for each deprivation level and bird) increased with the following order of the ranks: 2, 3, 4, and 1 respectively. The duration (Fig. 2) increased from 27 to 75 h of deprivation and the values of operated hens were larger than those of the controls. There was no simple correlation between duration and rank, but generally ranks 2 and 3 had larger mean durations than ranks 4 and 1. No dustbathing behaviour was observed at inspections during deprivation periods.

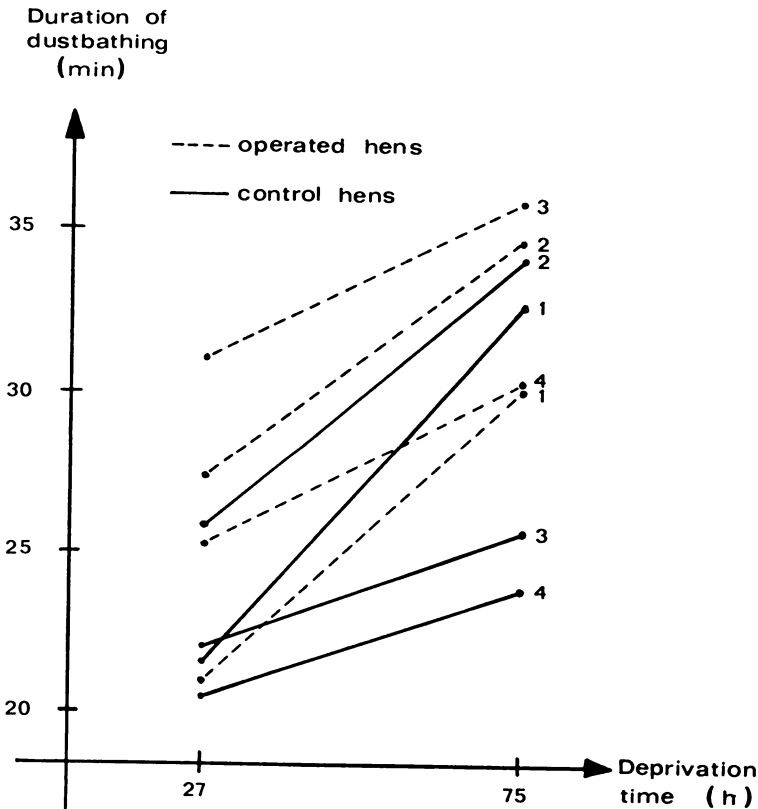


Figure 2. Duration of dustbathing after 27 and 75 h of deprivation for 4 uropygial-gland-extirpated and 4 intact hens. Number indicates rank in the hierarchies. Each point represents the mean of 10–15 observations.

Lipid content in the plumage

Lipid was obtained from both operated and control birds (Fig. 3) and there was no difference between the group means ($P = 0.55$). Filtration by the 8μ millipore filter reduced the values by approximately 20 % and again the difference between the groups was not significant ($P = 0.89$).

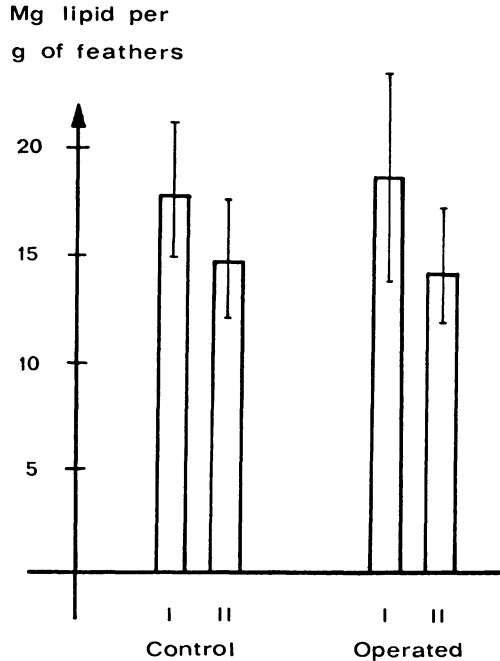


Figure 3. Feather lipid concentrations in operated and control hens. The columns show the mean concentration of lipids in 10 g of feathers from 4 uropygial extirpated and 4 intact hens. Values obtained by filtration through filter-paper (I) and through an 8μ millipore-filter (II) are shown. Standard deviation is indicated on each column.

DISCUSSION

This study has demonstrated a clear deprivation effect in uropygial-gland-extirpated hens. Like the intact birds in this study and those studied by Vestergaard (1981a) the uropygial-gland-extirpated birds increased their dustbathing tendency with the length of deprivation, i.e., latency decreased and duration increased. These findings are inconsistent with the lipid regulation theory, since there is no well documented way of in-

creasing the lipid content of the feathers except by oil from the uropygial gland. Also contrary to the theory the operated birds showed a higher tendency to dustbathe than the intact birds and this is difficult to explain. During the study, however, it was the impression of the observer that the plumage was more disorderly in operated than in intact hens, and since it is known that other external stimuli such as louse infestation and wet feathers increase the grooming behaviour of domestic hens (*Brown 1974*), it is likely that feather disorder may have a similar effect on dustbathing. The lack of uropygial gland lipid may have been the cause of the disorganized plumage in the operated hens.

Bobwhite quails dustbathe three weeks following uropygial-gland-extirpation, and the rate is the same as prior to uropygial-gland-extirpation (*Borchelt 1972* quoted by *Levine et al. 1974*). No information, however, is available about effects of increasing deprivation in these birds. Strictly keeping to the lipid regulation theory, uropygial-gland-extirpated birds should not dustbathe any more after having performed dustbathing a number of times, since that would have brought the amount of lipid below the threshold level for elicitation of dustbathing. *Levine et al. (1974)*, who tested various models of dustbathing and lipid regulation, which generally confirmed the theory, found that dustbathing also occurred for reasons not related to lipid regulation. This is further supported by a study of *Williams & Strungis (1979)* who found that during ontogeny of grooming behaviour in the domestic fowl dustbathing started some days before the oiling behaviour and before oil could be squeezed from the uropygial gland.

A further complication is that a supply of lipid may be coming directly from the skin of fowls (*Ishida et al. 1973*). Some accumulation of lipids may thus take place in the plumage of operated hens during dust deprivation periods, and such an effect might explain the observed deprivation-induced increase of the dustbathing tendency in the operated birds in the present study. However, contrary to what actually happened a lower dustbathing tendency should be expected in the operated than in the intact birds since the former were deprived of one of the lipid supplies. So even if we can accept that feather lipid may originate from sources other than the uropygial gland the present results do not confirm the oil regulation theory.

Besides lipids there may be other exteroceptive factors of significance for the long term regulation of dustbathing behaviour. Thus, it has been demonstrated that in Japanese quails dustbathing results in improved barb alignment, decreased amount of dandruff in the feathers, and pronounced drying and fluffing of the downs (*Healy & Thomas 1973*). Therefore feather disorder and accumulation of dandruff on skin and in the plumage may play a role in the long-term regulation of dustbathing and contribute to elevate the dustbathing tendency during experimental deprivation. Finally it may be that the regulation of dustbathing behaviour mainly depends on an internal regulation mechanism located in the central nervous system. More research is needed to elucidate the possible existence of such a mechanism.

No reduction in the lipid content of the plumage could be demonstrated in the uropygial gland extirpated birds. The values corresponded to those found in intact birds in a number of species including the fowl (*Bolliger & Varga 1960*). However, in the present intact birds plumage lipids might have been brought down to a "basic" level by dustbathing during the period with continuous access to litter preceding the collection of the feather samples.

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REFERENCES

- Benson, B. N. & M. W. Schein*: Factors influencing dustbathing in Coturnix quail. *Amer. Zool. Abstr.* 1965, 5, 196.
- Black, A. J. & B. O. Hughes*: Patterns of comfort behaviour and activity in domestic fowls: A comparison between cages and pens. *Brit. vet. J.* 1974, 130, 23—33.
- Bolliger, A. & D. Varga*: Cholestanol in avian plumage. *Aust. J. exp. Biol.* 1960, 38, 265—270.
- Borchelt, P. L.*: Dustbathing in Bobwhite Quail (*Colinus virginianus*): A Regulatory Model. Doctoral dissertation, Michigan State University 1972.

- Borchelt, P. L.*: The organization of dustbathing components in Bobwhite quail (*Colinus virginianus*). *Behaviour* 1975, 53, 3—4, 217—237.
- Borchelt, P. L. & L. Duncan*: Dustbathing and feather lipid in Bobwhite (*Colinus virginianus*). *Condor* 1974, 76, 471—477.
- Borchelt, P. L., J. Eyer & D. S. McHenry Jr.*: Dustbathing in Bobwhite quail (*Colinus virginianus*) as a function of dust deprivation. *Behav. Biol.* 1973, 8, 109—114.
- Brown, N. S.*: The effect of louse infestation, wet feathers, and relative humidity on the grooming behavior of the domestic chicken. *Poultry Sci.* 1974, 53, 1717—1719.
- Folch, J., M. Lees & H. S. Stanley*: A simple method for the isolation and purification of total lipides from animal tissues. *J. Biol. Chem.* 1957, 226, 497—507.
- Healy, W. M. & J. W. Thomas*: Effects of dusting on plumage of Japanese quail. *Wilson Bull.* 1973, 85, 442—448.
- Ishida, K., T. Suzuki, S. Kusuhara & M. Yamaguchi*: Influence of preen gland removal on the lipid over the plumage of roosters. *Poult. Sci.* 1973, 52, 83—87.
- Kruijt, J. P.*: Ontogeny of social behaviour in Burmese Red Junglefowl (*Gallus gallus spadiceus*). *Behaviour* 1964, Suppl. 12, 1—201.
- Levine, R. L., J. E. Hunter & P. L. Borchelt*: Dustbathing as a regulatory mechanism. *Bull. math. Biol.* 1974, 36, 545—553.
- Martin, G.*: Ueber Verhaltenstörungen von Legehennen im Käfig. Ein Beitrag zur Klärung des Problems tierschutzgerechte Hühnerhaltung. (On behavioural disturbances of laying hens in cages. A contribution to the clarification of animal protection problems in poultry-farming). *Angew. Ornithol.* 1975, 4, 145—176.
- Siegel, S.*: *Nonparametric Statistics for the Behavioral Sciences*. McGraw-Hill Kogakusha, Tokyo 1956.
- Simmons, K. E. L.*: Feather maintenance. In Thompson, A. L. (ed.): *A New Dictionary of Birds*. McGraw-Hill, New York 1964, pp. 278—286.
- Vestergaard, K.*: Dustbathing in the domestic fowl — Diurnal rhythm and dust deprivation. *Appl. Anim. Ethol.* 1981a, 7 (in press).
- Vestergaard, K.*: Behavioural and physiological studies of hens on wire floors and in deep litter pens. In (Ed. Fölsch, D.W.) *Das Verhalten von Legehennen*. (The behaviour of laying hens). *Tierhaltung* 1981b, 12 (in press).
- Williams, N. S. & J. C. Strungis*: The development of grooming behavior in the domestic chicken (*Gallus gallus domesticus*). *Poultry Sci.* 1977, 58, 469—472.

SAMMENDRAG

*Støvbadningsadfærd hos uropygialkirtel-extirperede tamhøner.
Effekt af støvdeprivation.*

Støvbadningsadfærd hos uropygialkirtel (gumpekirtel) extirperede („opererede“) og intakte Hvide Italiener høner blev undersøgt efter henholdsvis 27 og 75 timers støvmangel (deprivation). Når hønerne efter endt deprivationstid fik adgang til „støv“ i form af strøelse, blev en „kort“ latenstid og/eller en „lang“ varighed af støvbadningen taget som tegn på en forstærket trang til at udføre støvbadning. På grundlag af begge kriterier udviste de opererede høner en større støvbadningstrang end de intakte høner, og i begge grupper steg støvbadningstrangen fra 27 til 75 timers deprivationstid. På dette grundlag kunne eksisterende teorier gående ud på, at lipidmængden i fjerdragten regulerer støvbadningstrangen ikke bekræftes. Rangen i det sociale hierarki havde en indflydelse på både latenstid og varighed af støvbadningen, men der var ingen simpel korrelation mellem rang og støvbadningstrang. Der fandtes ingen forskelle mellem opererede og intakte høner i fjerdragternes lipidmængder efter 14 dages vedvarende adgang til støv.

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