Preening behaviour in laying hens: its control and association with other behaviours

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Abstract

Preening behaviour in laying hens has not been studied in detail, despite its association with dustbathing and possible role in feather pecking. After defining the components of the behaviour and determining the bout analysis criterion, this project examined preening behaviour and preen gland function, and how they were affected by bird age, beak treatment, and other external influences.

In layer pullets studied to 19 weeks of age, preening was evident within the first few days of life, and maintained an (albeit small) part of their daily behavioural repertoire. Times spent in preening, and in all other behaviours observed except dustbathing, were affected by bird age. The only effects of beak trimming (at 8 days of age) on preening were on times spent directed at the back (in sit posture) and preen gland (in stand and sit postures), with trimmed birds showing more than non-trimmed birds. Beak trimmed birds spent less time in litter directed activity than non-trimmed birds.

External influences such as frustration of feeding, feather pecking, presence of others (synchronisation), and type of floor substrate had little effect on preening, but variation in time spent preening between individuals was high. Preening observed during frustration of feeding could not be distinguished from normal preening, and so there was no evidence for classifying some preening as displacement behaviour. There were some differences in time spent preening between feather pecker and feather pecked status birds, but feather pecked birds did not preen more than non-feather pecked birds, as predicted. Groups of pen-housed layer pullets showed synchrony of preening at all ages observed, particularly when the proportion of time spent preening was high. Despite the association with dustbathing and the removal of stale feather lipids, times spent preening or dustbathing did not vary between birds housed on wire or litter floors. Peaks in preening and dustbathing were closely related in time, suggesting an association between them.

Preen gland morphology and histology were affected by bird age and size, but not by floor substrate. Older birds may be experiencing preen gland congestion, as judged by the solid consistency of preen gland contents. Feather lipid concentration was strongly affected by floor substrate when petroleum ether was used as the extractant, with some differences with bird age. Preen oil composition was affected by bird age and source of lipid (preen gland or feathers) but only 3 differences were detected with feather pecking and feather pecked status.

These findings suggest that time spent preening is variable between individuals and changes with age, but is not greatly affected by the external factors tested here. Preen gland development is closely related to bird age, but the only great effect of external influences appears to be that of floor substrate on feather lipid concentration. Presumed sebaceous secretions from the skin may also influence feather lipid level, feather lipid composition, and plumage odour.

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Declaration

I declare that this thesis and the work presented in it are my own. All assistance received has been acknowledged.

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Publications

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Chapter 1

Introduction

In scientific literature on domestic fowl, preening behaviour is rarely a central topic of papers in its own right, but remains largely as one behaviour of many that makes up an ethogram. Preening is often referred to as a 'comfort' behaviour, meaning one that is associated with body or plumage care, or stretching the muscles (van Rhijn, 1977; Nicol, 1989). In the current climate of concern over farm animal behaviour and welfare, it is difficult to see how preening in laying hens is relevant to welfare issues such as whether or not to ban the battery cage, and feather pecking and cannibalism. However, as will become apparent, preening is closely related to dustbathing, which would become functionally possible if recommended alternative systems to the battery cage, such as perchery/barn systems or enriched cages, became standard. Preening may also be associated with feather pecking, which can lead to cannibalism and death, major welfare concerns in the egg industry, particularly in alternative systems. The common practice of beak trimming amongst poultry producers and farmers may affect the accuracy of beak aim in preening.

This literature review will attempt to describe what is currently known about preening and its relation to other behaviours, the structure and function of the preen gland, preen oil, the effects of beak trimming, and the presence of red mites.

1.1 Preening behaviour

1.1.1 Preening defined

Preening behaviour is defined as "arrangement, cleansing, and general maintenance of the health and structure of the feathers by the bill...and...dressing of the plumage with

organic liquids (e.g. preen oil)" (Simmons, 1964). Head scratching with the foot, body shaking, and pecking at the legs/feet where they occur in close conjunction with feather manipulation are also associated with preening behaviour, since they too align and clean the plumage or skin. This is similar to the description given by van Rhijn (1977), who described preening in herring gulls as purely movements of the head and bill towards all parts of the plumage, with scratching and shaking being associated with, but not a part of, preening. In their study of the sandwich tern and the common tern (*Sterna sandvichensis* and *S. hirundo,* respectively), van Iersel and Bol (1958) included head and body shaking, stretching, and head rubbing in preening. Kruijt (1964) included head scratching but not body shaking in preening in red junglefowl (*Gallus gallus spadiceus*). Feather care is essential for maintaining good insulation and waterproofing properties plus ensuring preparation for flight.

Oiling behaviour, which is a component of preening designed to keep the feathers supple and aids in (but is not solely responsible for) waterproofing the plumage, (Fabricius, 1959), is the distribution of preen oil taken from the preen (uropygial) gland (see section 1.2) (Simmons, 1964). It is functionally different from other preening behaviour, as it is always initiated by rubbing the uropygial gland with the bill and head followed by swift movements of the bill over the plumage as the bird distributes the collected oil over the feathers (Kruijt, 1964; Simmons, 1964). Most oiling is directed at the breast, followed by the wings and flanks, and usually occurs twice a day, once shortly after lights on and once shortly before lights off (van Liere *et al*, 1991). After oiling behaviour, hens often then preen extensively at small localities of the plumage (van Liere *et al*, 1991). No literature could be found that distinguished between appetitive and consummatory preening, thus for the purposes of this project, all preening will be assumed to be consummatory.

1.1.2 Feathers

Preening behaviour is necessary to maintain the structure of feathers in working order. The domestic hen's adult plumage consists of flight feathers (remiges and rectrices), contour (body) feathers (Figure 1.1), plumules (down feathers) and filoplumes (hairlike

feathers) (Lucas and Stettenheim, 1972b; Deschutter and Leeson, 1986). The shaft (quill) of a feather is made up of the calamus, which is embedded into the follicle, and rachis. The rachis is the axis around which the vane is centred. The vane is made up of varying proportions of pennaceous (firm, closely-knit) and plumulaceous (downy) barbs, the latter which acts as insulation (Lucas and Stettenheim, 1972b), depending on the feather type.

The pennaceous portion of a feather consists of rows of parallel barbs, which project out from the vane, and are in turn covered in barbules, which link together with barbules from the adjacent barb with hooklets, like Velcro (Bradley and Grahame, 1960). Drawing the feathers through the bill during preening keeps the barbules interlocked and therefore resistant to airflow during flight and to water owing to its surface tension (Bradley and Grahame, 1960; Lucas and Stettenheim, 1972b). Some birds however, such as the ratites (ostriches, emus), have no interlocking structure to the barbules and this accounts for their lack of any pennaceous feathers (Bezuidenhout, 1999). Preening also keeps the feathers clean and free of ectoparasites.

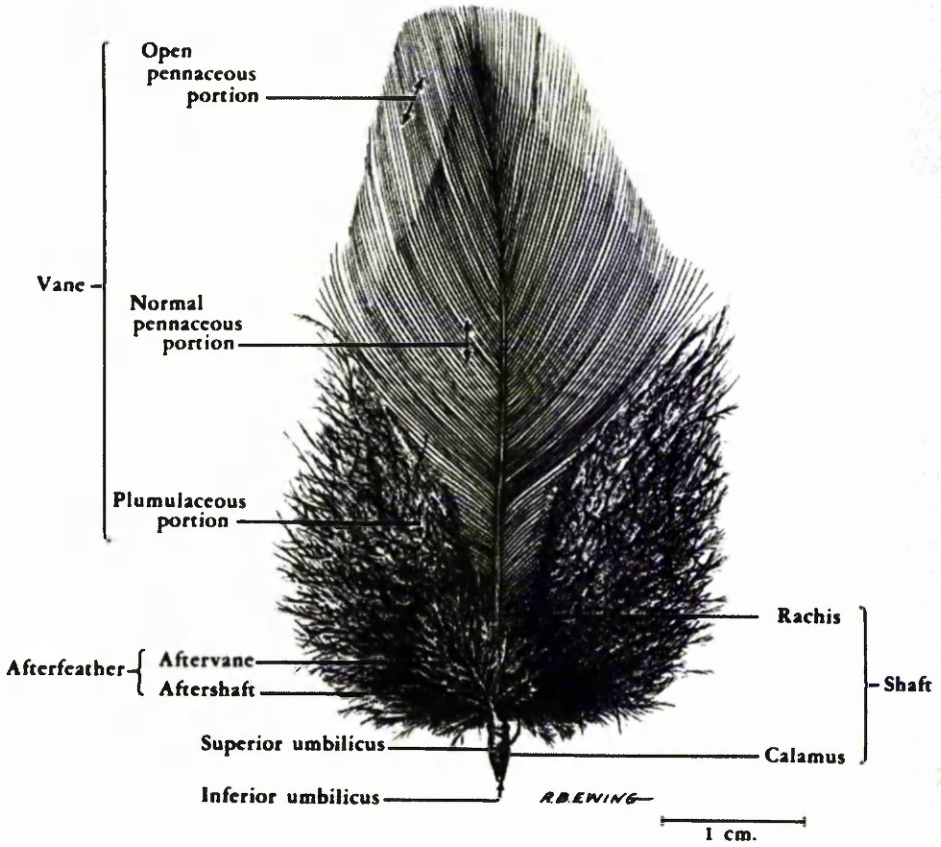


Figure 1.1 *A contour feather of a Single Comb White Leghorn chicken (reproduced from Lucas and Stettenheim, 1972b).*

1.1.3 Posture during preening

In ground-dwelling birds such as the domestic fowl, the postures adopted during preening and its associated movements are designed to prevent the plumage from coming into unwanted contact with the ground, which might soil and potentially damage the feathers (Cate, 1985). This contrasts to arboreal species, such as starlings or crows, which, during preening, frequently lower the tail and wings below the horizontal plane of the claws. Some methods that the domestic hen uses during preening to prevent touching its plumage to the ground substrate include maintaining a horizontal body posture and lifting its foot from under its wing for head scratching (Cate, 1985).

1.1.4 Age

Chicks as young as day-old express preening behaviour. In a study of male and female chicks observed from day-old to 5 weeks of age, preening the wing and body first occurred at day-old although oiling behaviour was not first observed until 28 days of age (Williams and Strungis, 1979). In junglefowl chicks, Kruijt (1964) first observed preening at day-old and oiling behaviour at 11 days of age, which was also the first day he managed to extract preen oil from the gland. In a study observing two different lines of domestic fowl composed of both male and female chicks, preening behaviour increased linearly over a 10 week observation period (Dawson and Siegel, 1967). In a study of different strains of chicks (one a White Leghorn x broiler cross, the others layer strains), proportions of preening in the Leghorn x broiler birds increased to 6 weeks of age and then remained constant until 10 weeks (Savory and Mann, 1997). The layer pullets increased the proportion of time spent preening until the period 13 to 16 weeks of age, and then remained fairly constant until 24 weeks of age. Preening is likely to increase over these periods due to the rate of feather development, especially in the wing feathers during the first two weeks of age (Williams and Strungis, 1979). Turkey pullets studied from 4 to 22 weeks of age also showed an overall peak in preening at 10 weeks (Sherwin and Kelland, 1998), probably again due to feather development. Feather loss during moulting also stimulates increased proportions of time spent preening compared to non-moulting birds (Webster, 2000).

1.1.5 Diurnal rhythms

In some early work on diurnal rhythms in laying hens, Wood-Gush (1959) recorded preening behaviour from time-lapse film during both summer and winter, under natural lighting conditions. There were no apparent seasonal differences other than the effect of shorter or longer day lengths on diurnal patterns in the frequency of preening, but peaks in preening seemed to occur just before lights off or just after lights on, as also found by van Liere *et al* (1991). This may be due to the reduction in more active behaviours (walking, pecking at litter) at these times and the increase in more static behaviours (standing, sitting in preparation for sleep) which are more conducive postures for

preening. There are no published reports on nocturnal preening in laying hens, although Sherwin and Kelland (1998) noticed that turkey pullets sometimes preened during posture changing after lights off.

1.1.6 Displacement preening

Displacement behaviours were first distinguished by Tinbergen in 1952 to refer to patterns of behaviour that occurred in apparently irrelevant situations (as cited in Manning, 1967). Displacement preening is expressed in a seemingly irrelevant context due to frustration or a conflict of motivational states (Hurnik *et al*, 1995), for instance during agonistic encounters (Kruijt, 1964) when presumably there is a conflict of whether or not to fight or flee. Frustration is caused by the interference of a motivated behaviour sequence (Duncan, 1970). Displacement activities can be confused with redirected activities which also occur in frustrating situations, but redirected activities are functionally similar to the motivated behaviour (Duncan, 1970). In a study on displacement preening in wild tern species, van Iersel and Bol (1958) considered preening to be displacement when a) it occurred out of context, for instance during or shortly after a conflict between brooding and escape; or b) the movements were more frantic than normal preening; or c) the composition (sites targeted or types of movements used) differed from normal preening. They described displacement preening as being disinhibited (and thus occurring) by the mutual inhibition of two conflicting behaviours (the disinhibition hypothesis). Duncan and Wood-Gush (1972a) characterised displacement preening in laying hens as being composed of a greater number of preen movements that were of shorter duration than 'normal' preening, and, during displacement preening, a greater proportion was spent targeting near body sites (breast, belly, shoulder) as opposed to the back, tail, and preen gland.

Displacement preening has been seen to occur during frustration of feeding behaviour. Wood-Gush (1959) noted that some preening in laying hens seemed to be motivated by competition at the food hoppers. Mild feeding frustration is more likely to stimulate displacement preening than more severe feeding frustration, which results in stereotypic pacing (Duncan and Wood-Gush, 1972b). Mild feeding frustration is stimulated by

giving birds a small number of training periods (in which they are fasted and then fed in a test cage repeatedly in order to train them to expect food when placed in the test cage) combined with short food deprivation periods, to result in low motivation to respond to a Perspex covered food dish (Duncan and Wood-Gush, 1972b). In Hyline Brown hens subjected to a 4-day fast (a relatively long period), fasted hens showed a significantly higher proportion of time spent preening compared to controls which the author thought indicated mild frustration (Webster, 1995). The deprivation period here was long, therefore one might expect pacing behaviour due to severe feeding frustration (Duncan and Wood-Gush, 1972b), but pacing behaviour was not recorded in this study. Proportions of time spent standing (which would presumably include pacing if it did occur) were slightly lower in fasted compared to control Brown hens. However, in Hyline W77 hens subjected to the same fast, there was no significant difference between fasted and control birds in proportion of time spent preening, yet fasted hens showed significantly higher proportions of time spent standing (and perhaps thus pacing) during the fast (Webster, 1995). W77 hens were thought to be generally more aroused during the fast than Brown hens, and thus perhaps this could account for the differences in preening and standing behaviour between these strains. Displacement preening may be caused not only by a lack of access to presented food, but unpredictable access to it. Hens subjected to uncontrollable additional access to food and light showed more preening behaviour than those hens that controlled their access to additional food and light by operant methods (Taylor *et al*, 2001). This higher level of preening may have been caused by frustration associated with unpredictable food access. In laying hens housed in battery cages, frustrated nest building due to a lack of suitable substrate may also be expressed as displacement preening (Mills and Wood-Gush, 1985), as feathers are the only material available which can be manipulated orally as a hen would do with nest building substrate.

1.1.7 Oral substitution

Preening behaviour may be used as an oral substitution for feeding behaviour. In restricted-fed growing broiler breeder females, both Kostal *et al* (1992) and Savory *et al* (1992) found that preening increased significantly when feeders and/or drinkers

(towards which redirected pecking was aimed) were removed, as one oral behaviour was substituted for another. Savory and Maros (1993) concluded that drinking, pecking at any non-food object (including litter) and preening can all substitute with each other as dominant post-feeding activities in restricted-fed broiler breeders. In broiler breeder females studied from day-old until 18 weeks and fed various restricted diets, preening behaviour was higher in the two most extreme restricted fed diet birds (designed to weigh 0.40 and 0.25 of *ad libitum* fed body weights) at 6 weeks but showed little difference at 12 weeks (Hocking *et al*, 1996). However, in a comparison of male layers and broiler breeders given *ad libitum* access to food, the broiler strain had significantly higher rates of food consumption but a significantly lower proportion of time spent preening and pecking (Hocking *et al*, 1997). This may be related to satiety effects of higher food consumption on oral behaviours or selection for energy conservation in the broiler strain, and/or a tendency to express more investigative or foraging behaviour in the layer strain.

1.1.8 Social facilitation and allelomimetic behaviour

Social facilitation is when 'the behavior of an animal reflectively increases the occurrence of the same behavior among its social partners' (Hurnik *et al*, 1995), whereas allelomimetic behaviour is 'the synchronisation of a behaviour by members of a group' (Savory, 1975). Definitions of these two phenomena do not always agree from study to study, for instance some authors use the term social facilitation to explain the effect of conspecific presence on subject behaviour, regardless of the conspecific's activity (Nicol, 1989; Nicol, 1995). In early studies on social facilitation, Tolman (1964) and Tolman and Wilson (1965) used the amount of weight gained by chicks reared in isolation, in pairs, or in groups to indicate social facilitation of feeding, however behaviour was not observed. Others specify that to be termed social facilitation *or* allelomimicry, conspecifics must be engaged in the same behaviour as the subject, without necessarily increasing its occurrence (Nicol, 1995; Palestis and Burger, 1998). Nevertheless, birds are undoubtedly affected by conspecific presence on their activities. Social facilitation of feeding behaviour in particular has been well-documented in laying strains of domestic fowl (Savory, 1975; Clayton and Carr, 1980;

Matsuzawa and Horikoshi, 1981; Keeling and Hurnik, 1996). Hughes (1971) demonstrated allelomimetic synchronisation of feeding in groups of broilers in all 9 h of the light period. Other behaviours that are synchronised include resting, sitting, standing, and head flicking (Webster and Hurnik, 1994).

Preening is influenced by both social facilitation, causing an increase in the behaviour, and allelomimicry, resulting in the synchronisation of the behaviour. Nicol (1989) found that, in laying hens, preening behaviour and body shaking significantly increased when a test bird was in close visual contact with its pen mates compared to visual contact at a metre distance or no visual contact at all. However, conspecifics behaviour was not recorded, thus social facilitation can not be positively inferred. Laying hens housed as pairs in battery cages with visual access to neighbours synchronised preening both within and between cages (Webster and Hurnik, 1994). A study of the common tern showed that as the density of the birds in a discrete area increased, so did the proportion of birds preening (Palestis and Burger, 1998). Pairs of Bengalese finches (*Lonchura striata*) were observed to synchronise preening significantly more than expected when in visual contact with each other than if the birds had been behaving independently of one another, which was suggested to facilitate protection from predators (Birke, 1974). In a study of a group of mynah birds (*Acridotheres cristatellus*), birds preened as a whole group after initiation of the behaviour by the most dominant bird (Nguyen-Clausen, 1975). Synchronising preening may have evolved as a method of defence, known as 'maintaining vigilance' (Fraser and Broom, 1997c), as during preening birds close their eyes and are therefore less aware of danger. By preening in groups, the likelihood of all birds having their eyes closed at once is low, and therefore the probability that a conspecific will spot a predator is higher than if a bird preened alone.

1.1.9 Allopreening

Allopreening occurs in some flocking species of birds, such as the red avadavat (*Amandava amandava,* a type of finch), which often allopreens its neighbour while perching (Evans, 1970). Harrison (1965) defined allopreening as meeting three criteria,

namely: a) containing similar actions to autopreening; b) areas allopreened are normally those inaccessible during autopreening, and; c) a soliciting posture is adopted by the bird being preened. Lill (1968) was hesitant to use the term allopreening with domestic chickens, because the movements he observed more closely resembled gentle feather pecking and were possibly related to aggression. This was based on noticing that aggressive pecking was often preceded or followed by gentle pecking, and that dominant birds allopreened subordinates more often than *vice versa*. However, Lill (1968) noticed that male-male allopreening was functionally more similar to autopreening than that seen between females, in that entire feathers were combed through the beak as opposed to just gentle nibbles or pecks. McBride (1970) suggested that non-agonistic pecking that was directed towards food particles on other birds' beaks or at feathers on birds' backs might be termed allopreening. With laying hens, there is a general failure for interactions with other birds' plumage to meet the criteria set out by Harrison (1965) to be classified as true allopreening, and it is more likely to be a form of feather pecking (see section 1.4). Wood-Gush and Rowland (1973) described some forms of hen-to-hen feather pecking as allopreening, but noted that birds did not adopt soliciting postures while being pecked, apart from during removal of food particles from around the mouth. Also, the pecking at other birds did not resemble most autopreening movements (i.e. nibbling at the feathers, combing through the bill), so, despite the terminology used by Wood-Gush and Rowland (1973), it seems unlikely that these movements could represent true allopreening.

1.1.10 Housing

Commercial laying hens are housed in a variety of environments ranging from the standard battery cage to barn (deep litter), perchery, and free range systems. Different environments vary in available space and provision of litter (see section 1.3), which may affect either the overall time spent preening or its components. Junglefowl chicks housed in rich (sand and grass covered flooring with perches) or barren (wire floors only) environment pens spent more time preening in the barren environment (Vestergaard *et al*, 1990). Black and Hughes (1974) observed three strains of layers from day old to 24 weeks of age housed in groups in cages with wire floors and in pens

with litter floors, and found that birds in cages showed more preening, head shaking and wing/leg stretching than those in pens, whereas birds in pens showed more dust bathing, wing flapping and ground scratching. Black and Hughes (1974) postulated that the higher frequency of preening in caged birds might have been due to feather damage caused by feather pecking or to frustration. If caged birds were at times unable to access the feed trough due to lack of space, this would be consistent with earlier reports of a relationship between displacement preening and frustrated feeding (e.g. Wood-Gush, 1959). Likewise, in the studies by Vestergaard *et al* (1990) and Black and Hughes (1974), in the more barren environment of cages there may have been substitution of foraging behaviour by preening, due to the lack of foraging substrate. Bareham (1972) found no significant effect of cage or litter floor (pen) housing on the amounts of time spent preening by two strains of laying hens, but these results may have been confounded by the fact that birds were housed individually in cages but grouped in pens. Thus, although birds housed on wire had visual access to other birds, there may have been more social facilitation of preening in group housed birds on litter.

Stocking density, and hence available space, seem to have little affect on preening in poultry. In two studies that tested effects of various stocking densities on behaviour of turkeys (Martrenchar *et al*, 1999) and laying hens (Carmichael *et al*, 1999), it had no effect on the proportion of time spent preening. In laying hens kept at different space allowances, birds kept at the highest space allowance (5630 cm^2 per bird) were closest together when they preened, and as space allowance decreased to 600 cm^2 per bird, the frequency of preening behaviour remained the same (Keeling, 1994b). Similarly, when two strains of domestic fowl were observed in a large outdoor enclosure, birds were found to be closest together when preening with both strains (Keeling and Duncan, 1991). Nicol (1987) compared individually housed laying hens at different space allowances, and found that those housed in small (847 cm^2) cages preened with the same frequency as those in large (2310 cm^2) cages. Jenner and Appleby (1991), however, found that the frequency of preening increased in groups of hens each housed for 1 week in different cage sizes, with increased cage space. As with synchronising

preening, preening in close proximity may be a defence mechanism against predators, both by increasing vigilance and by the dilution effect.

1.2 The Preen (Uropygial) Gland

1.2.1 Description of the gland

The preen gland, also called the oil or uropygial gland, is a bi-lobed structure that lies just below the dorsal skin surface at the base of the tail. It is the main cutaneous gland in birds, although there are sebaceous glands in the external ear passages and at the vent, and the skin itself can be regarded as a sebaceous gland (Thompson, 1964; Lucas and Stettenheim, 1972a; King and McLelland, 1975; Jacob and Ziswiler, 1982; Stevens, 1996). At the gland's posterior extremity, a small nipple-like structure with fine down feathers ('uropygial wick') protrudes from the skin, and it is from this that the bird collects preen oil with its bill and head (King and McLelland, 1975). The preen gland is not present in all bird species, such as ostriches and emus, which may be associated with their lack of hooklets on the barbules, resulting in the plumulaceous plumage (Bezuidenhout, 1999). Bustards, some pigeons and parrots also lack a preen gland (King and McLelland, 1975). The largest recorded gland, as a percent of body weight, is in the little grebe (0.61%) and the smallest is in fruit pigeons (0.02%), with diving and swimming birds having relatively large preen glands in general (Jacob and Ziswiler, 1982).

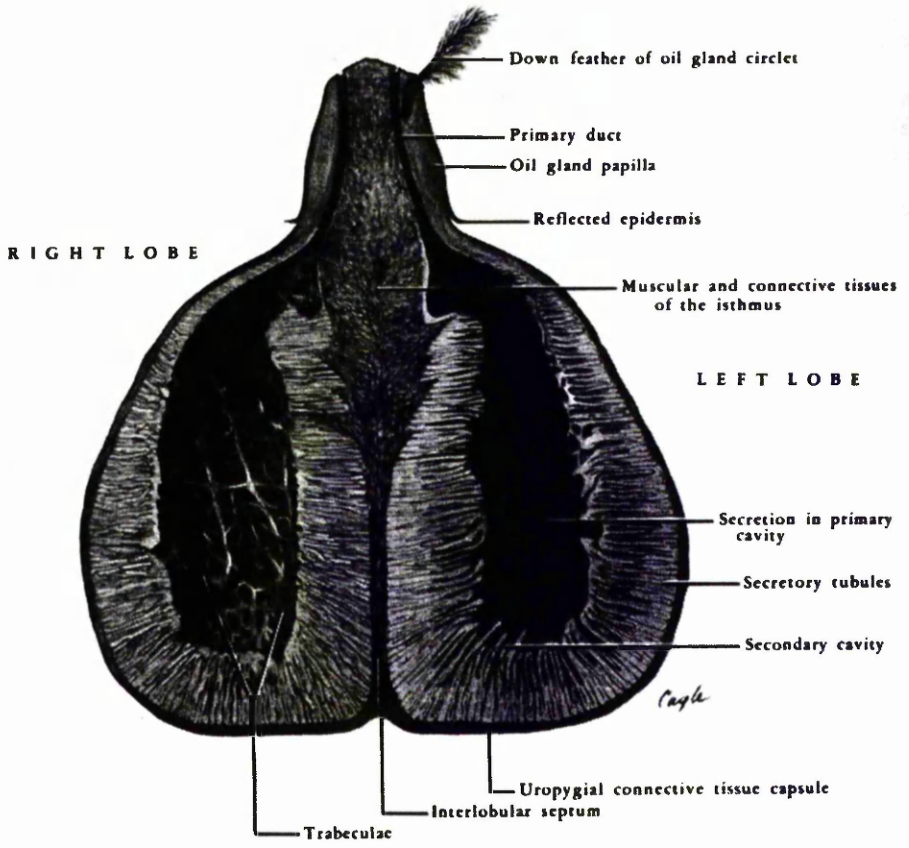


Figure 1.2 *Longitudinal cross section of a preen gland from a Single Comb White Leghorn chicken (reproduced from Lucas and Stettenheim, 1972b).*

In a longitudinal cross-section of the preen gland (Figure 1.2), the two lobes can be seen to be comprised of secretory tubules (capsules) and a cavity in which preen oil is stored. Between the two lobes is the connective tissue of the isthmus. The lobes drain oil into ducts either side of the isthmus, and as the pressure builds up from more oil production, the oil is forced out of the ducts of the papilla and onto the feather circlet, where the bird collects it with its beak (Jacob and Ziswiler, 1982) and then distributes it to the plumage. The tail muscles (Jacob and Ziswiler, 1982) and presumably pressure from manipulation by the beak or head may also have an indirect effect on preen gland oil expulsion.

1.2.2 Preen oil

The secretion from the preen gland, preen oil (also referred to as wax or lipid), is composed of a lipoidal (fat) fraction and a non-lipoidal fraction composed of proteins, inorganic salts and cell fragments (Rawles, 1960; King and McLelland, 1975). The lipoidal fraction is made up of monoester, diester and triester waxes (i.e. waxes which contain one, two, or three ester bonds), with the diester waxes being particularly prevalent in Galliformes (Haahti and Fales, 1967; Stevens, 1996). The diester waxes in Galliformes tend to be straight chain (unbranched) fatty acids of up to 24 carbon atoms with 1,2- and 2,3 diols (i.e. two alcohol portions on the first and second, or second and third, carbon atom of the fatty acid, respectively) (Haahti and Fales, 1967; Stevens, 1996). In a study of 14 different bird species, feather lipid made up 2 % (mean) proportion of dry feather weight, with the domestic fowl's feathers containing 1.2 % lipid (Bolliger and Gross, 1958; Bolliger and Varga, 1960). However, it is noteworthy that papers by Bolliger and Gross (1958) and Bolliger and Varga (1960) distinguish between feather lipid and preen gland lipid due to differences in their composition (particularly a lack of octadecanol, an 18-carbon chain alcohol, in feather lipid) and due to the presence of lipid on the plumage of the emu, a species that lacks a preen gland lipid. Feather lipids were thought to be a by-product of feather keratinisation (Bolliger and Gross, 1958; Bolliger and Varga, 1960), but they are more likely to be due to sebaceous secretions from epidermal cells of the skin (Lucas and Stettenheim, 1972a). A study by Ishida *et al* (1973) looked at plumage lipid on roosters and categorised two different types as 'granular' which contained fatty acids, and 'masses', which did not. The two types of lipid were thought to be derived from different sources, the skin (granular-type lipid) and the preen gland (mass-type lipid). In a study of hens with either their preen glands removed at 22 weeks of age or left intact, feather lipid levels did not differ between the two groups at 42 weeks of age, thus providing further evidence for different sources of oil on the plumage (Nørgaard-Nielsen and Vestergaard, 1981). It is possible that stale preen oil was still on the feathers after preen gland extirpation by the time feather lipids were measured, but the time period between extirpation and feather sampling (20 weeks), together with the provision of litter for dustbathing in between makes this unlikely. Lipids found on feathers may therefore be

a combination of preen oil applied to the plumage during oiling, and oil from sebaceous secretions.

1.2.3 Hormonal effects

Sexual maturity and seasonal variation in hormonal state also influence preen gland gross morphology and preen oil composition in birds. In young cockerels either castrated at 44 days of age or left intact, castrates had smaller preen glands relative to body weight than did controls at 86 days (Kar, 1947). Pigeons treated with artificial doses of estriol (a weak oestrogen) showed a significant decrease in preen gland weight relative to body weight and in the total amount of lipid obtained from the gland (Manna *et al*, 1983). In preen oil samples taken from chickens from 3 to 13 months of age, the composition of diols (alcohol portions of the lipid) changed, with C_{22} and C_{23} decreasing and C_{24} increasing as a percent of total diols, plus overall decreases in shorter chain ($C_{12} - C_{15}$) and increases in longer chain ($C_{17} - C_{20}$) fatty acids with age[1] (Kolattukudy and Sawaya, 1974). In male quails that were either castrated or left intact and maintained on a 16L:8D day length, intact males had a significantly higher concentration of C_{12} diol in preen oil, indicating a steroidal effect on the quality of preen gland secretion (Abalain *et al*, 1984). In mallards, the composition of preen oil was shown not to differ between male and female ducklings, or between ducklings and adult males (in which branched monoester waxes prevail), but in adult females there was a larger proportion of diester waxes than in ducklings or adult males (Jacob *et al*, 1979). Also, in adult females, the composition of preen oil changed over the reproductive period, indicating hormonal influences, whereas the lipid composition in males was shown to remain the same (Jacob *et al*, 1979). This seemingly conflicts with later work where both male and female mallards expressed seasonal variation in preen oil composition, with a significant rise in long chain fatty acids and decrease in short chain

[1] Molecules are listed either by the number of carbon atoms they contain only, e.g. C_{16}, or by both the number of carbon atoms and number of double bonds they posses (after a colon), where this is known, e.g. C16:0.

fatty acids during the breeding season, possibly coinciding with a rise and fall in levels of S-acyl fatty acid synthase thioesterase (Kolattukudy *et al*, 1985; Kolattukudy *et al*, 1987). The fatty acid content of preen oil may also be affected by diet, although preen oil synthesis is thought to occur mainly within the gland (Stevens, 1996). In chickens fed either a fat-free or 5 % corn oil supplemented diet, preen oil composition of the supplemented diet birds included branched pentacosanoic (C25:0), octadecanoic (C18:0) and nonadecanoic (C19:0) fatty acids, all of which were absent in preen oil from the fat-free diet birds (Apandi and Edwards, 1964). There were also large decreases in the amount of straight chain fatty acids (n-octadecanoic, (C18:1), n-nonadecanoic (C19:1) and n-eicosanoic (C20:1)) in the supplemented diet birds (Apandi and Edwards, 1964).

1.2.4 Preen oil function

The function of preen oil is varied. It serves to keep the feathers aligned and in good condition and contributes to waterproofing the plumage. The wax acids and alcohols have antimicrobial properties which serve to keep the plumage, skin and nest relatively free of bacteria and fungi (Jacob *et al*, 1979; King and McLelland, 1984; Stevens, 1996). Jacob *et al* (1979) suggested that the differences in branched and unbranched monoester waxes between male and female mallards, and between females and ducklings, might affect the odour of preen oil and hence preen oil might act as a pheromone. Certainly odour can influence bird behaviour: goslings given previous access to warming boxes impregnated with goose oil and then given a choice test between boxes with an unfamiliar odour or goose oil always chose the familiar scented boxes (Würdinger, 1982).

A common belief is that, due to its proportionally large size in aquatic birds, the main function of the preen gland is to supply oil to waterproof the plumage. However, while its secretion is undoubtedly helpful in repelling water, the gland is also proportionally large in some other non-aquatic species such as Galliformes and the oilbird (Rawles, 1960; Stevens, 1996). Additionally, despite possessing a relatively large preen gland, the cormorant's flight feathers get wet through after entering the water (Jacob and

Ziswiler, 1982). After the preen glands of ducklings were removed immediately after hatching, their plumage remained dry when the ducklings were allowed to swim (Fabricius, 1959). The main factor in waterproofing was thought to be attributed to the interlocking structure of the barbules of the plumage, which water cannot penetrate (Fabricius, 1959). Ducklings handled with bare hands or soiled with food and then made to swim immediately got wet through upon swimming, but preening restored the interlocking structure of barbules and the plumage was waterproof once again (Fabricius, 1959). The combination of preen and epidermal lipid production alongside the structure of interlocked barbules which is maintained by preening prevent water penetration and all contribute to keeping the plumage waterproof (Fabricius, 1959; Lucas and Stettenheim, 1972a; Lucas and Stettenheim, 1972b). Chickens artificially wetted with water show significantly more time at the preen gland and in grooming compared to a group of dry birds, or compared to pre-wetting behaviour values in the same group of birds (Brown, 1974), indicating that water penetrating the plumage may stimulate application of preen oil, which would then reinstate the integrity of plumage by its properties and method of application (preening).

1.3 Dustbathing

Bird species carry out bathing behaviour, in water or dust. Dustbathing incorporates tossing floor substrate onto the plumage, rubbing, and then shaking to remove the litter particles (for illustrations, see van Liere, 1992b). The laying hen performs all its baths in dust (referring to any dry, particulate substrate) where available (Simmons, 1964), but for many this is thwarted due to housing in cages without floor litter, where 'sham' or 'vacuum' (non-functional) dustbathing is often carried out. Development of dustbathing behaviour in adults is not dependent on experience with a suitable substrate as chicks (Vestergaard *et al*, 1990). In pullets housed in either (litter) pens or cages from day old to 24 weeks of age, those housed in pens showed significantly more dustbathing than those in cages, but caged birds still expressed the behaviour to some

degree, showing that it is at least partly motivated internally and not purely due to the stimulus of a suitable substrate (Black and Hughes, 1974).

1.3.1 Function of dustbathing

The function of dustbathing is to remove stale oil (derived from the preen gland and, presumably, skin) (van Liere and Bokma, 1987; van Liere *et al*, 1991; van Liere, 1992a) and ectoparasites from the plumage (Simmons, 1964). This helps to keep the plumage in good condition, although other factors such as damaging featherpecking may mask dustbathing's beneficial effects on plumage quality (Abrahamsson *et al*, 1996). Different types of dustbathing substrate (e.g. woodshavings or sand) remove varying quantities of lipid from feathers, depending on how deeply they penetrate the plumage (van Liere *et al*, 1990; van Liere, 1992a). Birds deprived of dustbathing substrate and then given either sand or woodshavings spent more time dustbathing on woodshavings than on sand in order to achieve similar reduced feather lipid levels (van Liere *et al*, 1990). This may be because woodshavings do not penetrate to the downy part of the plumage as easily as sand, or because sand may be a better adsorbent than woodshavings for lipid.

1.3.2 Relationship between dustbathing and preen oil

Dustbathing is partly induced by build up of preen oil on plumage (Levine and Hunter, 1974). The effects of dustbathing substrate deprivation can be seen early in life, as development of dustbathing appears in chicks aged 8 days or less (Hogan *et al*, 1991). In a study of red junglefowl chicks, the number of vertical wing shakes during dustbathing increased with longer prior deprivation of substrate, and it was suggested that this may be due to a build up of preen oil on the plumage (Hogan *et al*, 1991). Stale preen oil accumulates in the absence of dustbathing material as the bird continues to carry out oiling behaviour (Borchelt and Duncan, 1974). In hens deprived of dust for 33 days, the concentration (mg per g feathers) of lipid extracted from feathers increased significantly compared to pre-deprivation levels and control birds' feathers, but were reduced to their original levels after just two days of access to dustbathing substrate

(van Liere and Bokma, 1987). During both short-term (33 days) and long-term (21 weeks) deprivation of friable substrate, laying hens show sham dustbathing, as the motivation to dustbathe is still present in the absence of an appropriate substrate due to the build-up of lipids on the plumage (van Liere and Bokma, 1987; van Liere and Wiepkema, 1992). During deprivation of litter substrate, Borchelt (1975) found a decrease in the initial components of dustbathing (scratching and pecking at the floor) and a significant increase in tossing and rubbing movements. It is unknown whether or not any feather lipid is removed during sham dustbathing.

More specifically, it is thought that it is the presence of stale lipid on plumage, as opposed to the total amount of lipid, that affects the duration of dustbathing in hens. The application of either fresh or stale preen oil to the plumage resulted in a significantly longer duration in dustbathing in birds with stale lipid than in those with fresh lipid or control birds (van Liere *et al*, 1991). However, it may not be purely the build up of preen oil that affects dustbathing. In a comparison between uropygial gland extirpated hens versus controls, extirpated hens had a shorter latency to begin dustbathing after deprivation of dustbathing substrate, and duration of their dustbaths were longer (Nørgaard-Nielsen and Vestergaard, 1981). Levels of feather lipid extracted from these two groups of birds did not differ significantly 20 weeks after preen gland removal, suggesting the presence of another lipid on the extirpated hens, perhaps that derived from epidermal lipid production (Lucas and Stettenheim, 1972a). Mammals prevented from bathing also suffer from oily fur due to a build up of skin lipids (Borchelt *et al*, 1976; Barber and Thompson, 1990).

1.3.3 Thermoregulation

A build up of preen oil affects thermoregulatory properties of feathers, by matting the plumulaceous part of the feathers. Schein and Statkiewicz (1983) proposed that in Japanese quail, those birds with access to dustbathing substrate and therefore having a better plumage condition were more able to withstand cold weather than those without dustbathing substrate. In birds deprived of dustbathing substrate for 33 days, the downy parts of feathers became less fluffy (van Liere and Bokma, 1987). After two days

access to dustbathing substrate, the fluffiness of the downy parts of feathers improved significantly. In a comparison between different dustbathing substrates (peat, sand and wood shavings), the substrate that gave the lowest concentration of feather lipids upon extraction and the fluffiest plumage (peat) was also that which gave the lowest plumage surface temperature, indicating that there was less heat loss through the plumage of birds housed on peat (van Liere and Siard, 1991; van Liere, 1992b).

1.4 Feather pecking

The term feather pecking refers to a wide variety of non-aggressive pecking behaviours that birds direct towards conspecifics: pecks directed at litter or particles of food on the plumage or beak, which usually results in little or no feather damage; feather pulls that sometimes remove feathers and usually cause the recipient to withdraw; and tissue pecking at exposed skin that may draw blood and can lead to severe injury or death (Savory, 1995). Keeling (1995) defined feather pecks as either gentle (little or no feather damage) or severe (forceful pecks or pulls that usually result in recipient withdrawal). The confusion between gentle feather pecks and allopreening has already been indicated in section 1.1.9. In addition, in a study of the relationship between feather pecking and dustbathing, Vestergaard *et al* (1993) described some forms of pecking as allopreening, which may at times have resembled feather pecking as they involved pecks at the plumage. It was noted that pecking was often difficult to identify as either allopreening or feather pecking (that which can cause feather damage), so when in doubt the pecking was referred to as allopecking. This highlights the indistinct nature of the less severe forms of pecking with regards to their function.

Preening and oiling behaviour may influence feather pecking. In a study of the relationship between feather pecking and feather eating, McKeegan and Savory (2001) found that when caged birds were presented with washed or unwashed feathers, birds preferred to eat unwashed ones, which the authors suggested indicated a preference for feathers coated in preen oil, or an aversion to washed feathers for some reason. In a

study of feather pecking and cannibalism in layers, Keeling (1995) found that severe feather pecks were usually directed at feeding or preening birds. This may have been due to targeted birds being stationary and therefore easier to peck at as opposed to an attraction to birds specifically because they are preening. On the other hand, preening may attract pecking birds' attention towards the feathers of pecked birds. Likewise with Savory and Griffiths' (1997) study of growing bantams, where those birds that showed more preening movements received more feather pulls, and Savory and Mann's (1997) study of layer pullets, where birds that were pecked were usually performing stationary behaviours such as standing and preening. When observing red junglefowl housed either with or without floor substrate (sand, grass woodshavings), it was noted that more feather pecks tended to occur during periods in which dustbathing and/or dustbathing intention movements occurred in both groups of birds (Vestergaard *et al*, 1993). This may be because dustbathing birds are an easy target, or due to the contrast of substrate particles on the plumage, where it was present. No other behaviours were observed so it was not possible to infer relationships between feather pecking and other behaviours such as feeding or preening.

Some feathers that come loose during preening are consumed by the preening bird (pers. obs.), which may then result in feather pecking and feather eating being directed at other birds. In a study with Hisex pullets, the only group to eat plucked feathers also developed pecking behaviour (Savory and Mann, 1997). By looking at (indigestible) feathers in faecal droppings as an indicator of feather eating, McKeegan and Savory (1999) found that pecking damage scores were correlated positively with proportions of droppings containing feathers, and negatively with the number of loose short feathers on the pen floor, implying that feather eating is involved in the development of feather pecking.

1.5 Beak trimming

General commercial practice in the management of laying hens includes beak trimming the birds in order to prevent feather pecking and cannibalism. Beak trimming is commonly carried out at the hatchery or within the first ten days of life (Hughes, 1984; Hubbard ISA, 1998), although no regulations prevent beak trimming throughout the lifetime of the bird (MAFF, 1996). Beak trimming in the UK must not remove more than one third of the upper and lower mandibles, is performed without anaesthetic (MAFF, 1996), and is most commonly performed using a cauterising heated blade debeaker. This consists of a choice of holes of varying diameters through which the mandibles are placed and a hot moving blade that passes up and down past the openings, both cutting and cauterising simultaneously. Beak trimming affects pecking by reducing the sharpness of the beak and accuracy of pecks and by making pecking painful (at least in birds beak trimmed at 5 weeks of age or older) (Gentle, 1986a; Hughes and Gentle, 1995). Less feather pecking results in better plumage condition and therefore better thermoregulation, which in turn leads to better feed conversion efficiency (Hughes and Gentle, 1995).

Trimming the beak, which is abundantly supplied with nerve endings, involves pain to the bird and reduces sensory feedback from the beak (Hughes and Gentle, 1995). The trimming process causes acute pain due to creation of the wound, and chronic pain may be experienced as a result of neuroma formation in the beak stump. In a study by Gentle *et al* (1990), those hens that had been beak trimmed at 17 weeks of age showed a reluctance to drink warm water (45° C) compared to controls up to 6 weeks post-trimming, suggesting chronic hyperalgesia. Trimming adult birds can also reduce both food intake during the first post-operative week and feeding efficiency (number of pecks per gram of food eaten) during the first 4 days post-operatively and therefore have a compounding effect on body weight (Gentle *et al*, 1982). Beak trimming birds at 7 weeks of age has been shown to chronically reduce the number of eggs produced per hen housed and mean egg weight, when production was recorded from 19 to 58 weeks of age, however mortality, food intake and feed per dozen eggs were lower in beak trimmed versus non-beak trimmed groups (Bell and Adams, 1998). The benefits of

beak trimming are evident when cannibalism outbreaks occur. In a comparison between deep litter and cage systems, those birds that had not been beak trimmed when cannibalism erupted showed higher levels (4 % – 9 %) of mortality than those that had been beak trimmed (2 % – 3 %) (Appleby *et al*, 1988). Grigor *et al* (1995) found that female turkeys that were beak trimmed at 1, 6, or 21 days of age and studied over 12 weeks showed significantly less pecking at other birds than did untrimmed controls. If beak trimming is carried out at a young age, birds seem to experience less chronic pain. Although layer chicks that were beak trimmed at 1 or 10 days of age showed significantly less beak related behaviour in the first week after trimming than did control chicks, this difference was greatly reduced by 5 weeks after trimming (Gentle *et al*, 1997). Weight gain was similarly reduced in trimmed compared to control chicks in the first week after trimming, but were statistically similar by the second week (Gentle *et al*, 1997).

Partial amputation of the sensitive beak structure can be expected to affect preening. Beak trimming has been shown to decrease preening, and other beak-related behaviour, in birds trimmed at both 1 and 10 days of age with either hot or cold cut methods, during the first post-operative week (Gentle *et al*, 1997). Hot cut birds were still showing less preening behaviour after 3 weeks than either cold cut or controls. In a study with birds trimmed at 16 weeks of age, preening was significantly reduced compared to pre-treatment values, both immediately and in the 5 weeks after trimming (Duncan *et al*, 1989). Compared with sham operated controls, these beak trimmed birds expressed less preening behaviour in both the short term and long term. In a study of female turkeys, preening was significantly reduced in birds beak trimmed at 1, 6, or 21 days of age compared to controls up to 12 weeks post-trimming (Grigor *et al*, 1995). Although beak trimming is beneficial in reducing feather pecking damage and cannibalism, the consequential reduction in preening may have detrimental effects on bird welfare.

1.6 Red mites

The poultry red mite, *Dermanyssus gallinae*, is a hematophagous (blood sucking) nocturnal mite less than 1 mm long that thrives in the warm humid conditions of the commercial poultry house (Levot, 1997; Nordenfors *et al*, 1999; Nordenfors and Höglund, 2000). They are prevalent in all types of poultry housing, although they appear to be less problematic in cage systems (Höglund *et al*, 1995). This is probably due to the metal surfaces of cage systems being easier to clean, as opposed to barn and perchery systems that contain more wood in which mites can hide. Poultry mites can survive without feeding for up to nine months, so may persist even if a house is depopulated for several months (Nordenfors *et al*, 1999). They are a serious pest in the laying industry as they can cause anaemia and even death (Abrahamsson *et al*, 1998), especially in young birds (Kirkwood, 1967) and are a likely source of stress. Blood loss in laying hens due to red mite infestations can lead to a drop in egg production, incurring further costs on producers (Maurer *et al*, 1993).

Preening is possibly influenced by red mites due to irritation they would cause while feeding on the bird. In a review of literature on maintenance behaviour (grooming, bathing, scratching and stretching), Cotgreave and Clayton (1994) found that those species that had higher louse infestations spent relatively more time grooming. In a comparison between chickens infested or not with Mallophaga lice, the frequency of preening was significantly higher in the infested groups (Brown, 1974). In a study on turkeys artificially infested with ticks, more than 90 % of the ticks were thought to be ingested during preening (Ostfeld and Lewis, 1999), indicating that this behaviour is an effective method of pest control. Similarly, when lice were glued onto the plumage of feral pigeons, preening effectively removed lice (Rózsa, 1993). Beak trimming the pigeons significantly reduced their ability to remove lice from the plumage during preening (Rózsa, 1993), which could have serious consequences on parasitic load and therefore bird health. Beak trimming laying hens may therefore affect their ability to control red mite load through precise preening. As well as preening, birds may take evasive behaviour (similar to gadding in cattle) (Hart, 1992) in order to avoid the mites.

In alternative systems to cages where birds have more space in which to move, birds may flap or fly about in darkness (when red mites are active) in order to escape mite irritation, resulting in the birds colliding with each other or stationary objects and suffering injury.

Preen oil may be an attractant to (or partially fed upon by) red mites, which would suggest that those birds without access to dustbathing substrate, as in cages, would have a higher infestation rate due to preen oil build-up. *In vitro* studies on red mites showed that mites would only feed through bird skin (chick or quail) and not through mammal skin or artificial membranes, but would feed on bird or mammal blood (Kirkwood, 1971). Another study on red mites fed on chicken blood through a variety of natural and artificial membranes revealed that mites preferred unwashed over washed chicken skin, but preferred a synthetic membrane even more than chicken skin if it was impregnated with feather extract (Zeman, 1988). This suggests that something other than (or as well as) preen oil attracts and/or is fed upon by red mites, such as lipid secretion from the skin.

1.7 Aims and outline of the thesis

Preening behaviour in the domestic fowl, its control and association with other behaviours, is poorly understood. The aims of this project were to describe the development of the behaviour in the growing bird, plus to examine how some external factors influenced preening in adults. The function of the preen gland in different contexts was explored, due to its association with preening.

The experimental work of this project is preceded by a chapter on the materials and methods used throughout (Chapter 2), which includes a pilot study to determine which behaviours were recorded in all further experiments, and describes how the bout criterion interval was assessed. The experimental work is divided into three chapters. A long-term study into the effects of beak trimming in layer pullets on preening and other behaviours is given in Chapter 3. Chapter 4 describes 5 experiments that examine

a variety of external influences (frustration, feather pecking, presence of conspecifics, and type of floor substrate) on preening and other behaviours. In Chapter 5, preen gland function is examined by exploring gland morphology and histology, the concentration of lipid found on feathers, and the composition of preen oil, with regards to bird age, floor substrate, and feather pecking status. A general discussion comparing behaviour results across all relevant experiments, and linking behaviour to preen gland function, is in Chapter 6. Appendix A details the data collected in each study, while Appendix B is supplementary data to Chapter 3. Appendices C – E are aids in understanding the allelomimetic analysis described in Chapter 4. Appendix F gives an example of a gas chromatogram.

Chapter 2

General Materials and Methods

Many methods employed in this thesis were used repeatedly. The following information details materials and techniques used regularly, in order to avoid repetitious explanations. The main methods used for each experiment are laid out in Appendix A.

2.1 Subjects

Birds used were predominantly ISA Brown or Lohmann Brown. One study used Lohmann x Single White Leghorn (LSL) hens (a light hybrid cross) (Experiment 3). Birds were brought in at day old, at 18 weeks of age (point of lay), or at end of lay for study. All birds brought in at 18 weeks of age were beak trimmed at the rearing site. The only birds left intact upon arrival at the study site were day-old chicks used in Experiments 1, 4 and 6c, and the LSL end-of-lay hens.

Individual birds were identified using wing tags, or different coloured leg rings. Three different leg ring sizes (6.4, 8, and 14 mm) were used to accommodate leg growth.

2.2 Husbandry

2.2.1 Housing

General commercial practices were carried out with regards to heating, lighting and feeding and these are detailed in each chapter. Birds were not necessarily studied immediately after arrival from suppliers, but may have been part of commercial stock before being removed and placed into new housing for study (Experiments 2, 3, and 5a). Subject birds were housed in individual cages, individual pens or group pens. Cages were provided with nipple drinkers and trough feeders. Individual pens had nipple

drinkers and trough feeders, and either wire or litter floors. Group pens had one bell drinker and one food hopper, and litter floors. Specific cage and pen dimensions are given in the relevant sections. Apart from the pilot study described in section 2.3.1, individual cages or pens used during remote observations had mesh roofs through which video recording could take place (see section 2.3.2). These were raised off of the floor so that trays or litter could be placed beneath to collect droppings. Cage or pen walls were made of solid partitions, excluding those used in section 2.3.1. For direct observations of birds housed in individual cages (Experiment 3), cage fronts and backs were made of wire, but had solid sides. In group pens (Experiments 1, 4 and 6c), walls were constructed of solid wood to 10 cm higher than bird head height with nylon mesh above.

2.2.2 Bird growth

Birds were individually weighed on a regular basis using a top loading 6 kg balance for chicks or 15 kg balance for pullets and adults. Although bird weight never exceeded 3 kg, the 15 kg balance had a larger pan which made balancing easier for bigger birds. Details of the weighing schedules are given in each relevant section.

2.2.3 Beak lengths

The upper and lower mandibles of birds were measured in order to monitor the effect of beak trimming on beak morphology, and to assess this as a factor in differences in preening behaviour. Mandibles were measured from the beak tip to the back of the 'gape' when the mouth was held slightly open, using a pair of callipers, to the nearest 0.1 mm.

2.2.4 Pecking damage scores

Birds in some experiments were assessed for pecking damage to the plumage. This consisted of inspecting the neck, back, breast, wings, and tail (including the belly), and assigning a score, as based on the method by Savory and Mann (1997). These were: 0, no damage; 1, slight feather damage/loss but no bare skin; 2, damage with < 1 cm^2 bare

skin; 3, moderate damage with up to 5 x 5 cm^2 bare skin; 4, severe damage with > 5 x 5 cm^2 bare skin.

2.3 Behaviour measurements

2.3.1 Pilot study

An initial pilot study was conducted to practice video recording and data collection methods. This was also necessary to determine details of what constituted preening and therefore what behaviours to record during all subsequent studies (an ethogram). A total of 12 individually caged Lohmann Brown hens aged 18 weeks were housed in a small battery of cages (3 tiers x 9 cages per tier x 2 sides). Groups of 6 birds were placed side by side in the middle tier of each side of the battery. Each cage had a floor area of 1833 cm^2 and 2 nipple drinkers and a trough feeder. Cage sides were made of wire mesh so hens were not visually isolated. Hens had *ad libitum* access to food, and 8 h of light, increased to 10.5 h (08:00 – 18:30) by age 20 weeks when recording took place. Each hen was video recorded for one hour between 10:00 and 13:30 h, with a black and white CCTV camera (model WV-BP310/B). The camera was mounted on a tripod in front of the cages, so that two neighbouring hens were recorded simultaneously through the plastic bar cage front. The camera was connected to a time-lapse video cassette recorder, which also superimposed the time and date onto the tape (model AG 6720, both camera and recorder by Panasonic, Germany).

Initial observations of the video-recorded data, together with published descriptions of preening (Kruijt, 1964; Simmons, 1964; van Rhijn, 1977) revealed that this behaviour was made up of three types of mandibulations. These were gentle pecks at the plumage (peck), high frequency nibbling movements of the bill, usually at the base of the feathers (nibble) and drawing the feathers through the partly closed bill (comb). The body sites were divided up into the back (which included the back of the neck and rump), wings (including the shoulders), flanks, breast (which included the front of the neck and belly), tail and preen gland, (Figure 2.1), according to van Liere *et al* (1991).

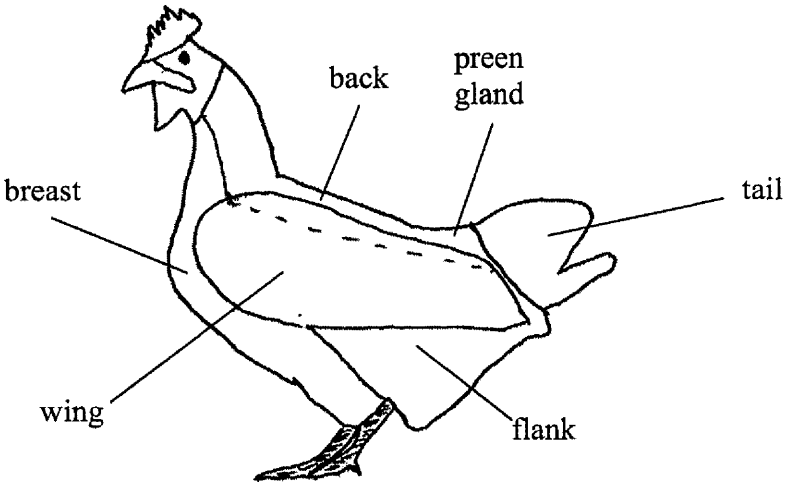


Figure 2.1 *Body sites identified during preening.*

Body shaking (van Iersel and Bol, 1958) and head scratching (Kruijt, 1964) had been described as integral parts of preening, so these were recorded as well when they occurred within 60 sec of a preening movement.

In one hour of recording, hens spent on average 4.1 % of time in preening, associated body shaking or head scratching. Of the total time spent in these behaviours, hens spent (mean) 16.3 % at the breast, 25.6 % at the wings, 12.7 % at the flanks, 12.0 % at the back, 6.2 % at the tail, 1.0 % at the preen gland, and 1.5 % in body shaking/head scratching. Due to difficulty in determining what body site was being preened when the hen's head was obscured from the camera, a mean 24.7 % of preening was 'unidentified'. Therefore, proportions of time spent at the body sites given above will be somewhat inaccurate.

From these observations, it was clear that recording each preening site plus different forms of mandibulation (peck, nibble, and comb) was difficult to visualise and record swiftly enough without missing a change in behaviour. It also broke down what already constituted a very small amount of the repertoire of laying hen behaviour into even smaller fragments, making comparisons between birds or treatments difficult due to many zero values. Likewise, the body sites were not divided further into more discrete

sites (for instance, dividing the wings into outside and inside wing). For the sake of accuracy, all following studies recorded the body sites as originally determined but the different types of beak manipulations were ignored. As well as body shaking and head scratching, hens were also seen to show wing flapping and pecking at the legs or feet during, shortly before, or after preening. It was therefore decided that these should also be recorded where they fell in close association (< 60 sec) with preening. A general ethogram of behaviours was generated from this information (Table 2.1).

2.3.2 Sampling and recording by remote observation

The use of video recording was implemented for *ad libitum* sampling in the pilot trial described in 2.3.1 and for Experiments 2, 5a, and 5b in Chapter 4. This ensured that preening behaviour, which was relatively infrequent, was not missed no matter how short the duration. It was also useful in case of behavioural recording mistakes during transcription from video to computer, in which case the exact segment could be replayed, or for further data collection of other behaviours. From the videotapes, all preening and dustbathing behaviour were continuously recorded to give precise durations.

2.3.3 Sampling and recording by direct observation

Direct observations were made from a seated position outside the pen or cage using scan sampling (in which a group or individual was scanned at regular intervals) (Experiments 1 and 4), or a combination of scan sampling with behaviour sampling (an individual was watched for a particular behaviour; Martin and Bateson, 1993) (Experiment 3). Scan sampling was recorded instantaneously ('on the beep'; Martin and Bateson, 1993) at fixed intervals. Behaviour sampling of preening was recorded continuously. Where each technique was used is indicated in the appropriate section.

Table 2.1 *General ethogram of mutually exclusive behaviours used, and in which experiment each behaviour was recorded (no behaviour was recorded in Experiments 6a, 6b, and 6c).*

	Experiment number				
Behaviour (defined where necessary)	**1 & 4**	**2**	**3**	**5a**	**5b**
Preening[1] (manipulation of feathers with bill, while standing or sitting)	✓	✓	✓	✓	✓
Comfort associated with preening:					
Body shaking	✓	✓	✓	✓	✓
Head scratching	✓	✓	✓	✓	✓
Pecking at leg/foot	✓	n.o.[2]	n.o.	✓	✓
Wing flapping	✓	n.o.	n.o.	✓	✓
Other comfort:					
Bill wiping	✓		✓		
Stretching	✓		✓		
Head out (head, or head and shoulders, held outside cage front)		✓			
Drinker directed (pecking at water source)	✓	✓	✓		
Feeder directed (pecking at, or head over, feed)	✓	✓	✓		
Walking (walk or run)	✓	✓	✓		
Dustbathe (starts with first vertical wing shake or dust toss, ends with first body or wing shake, or 5 min non-dustbathing behaviour)[3]	✓	n.o.	✓	✓	✓
Spot peck	✓	✓			
Non-aggressive peck (gentle pecks, often directed at the beak or at particles on the plumage, or feather pecks)	✓		✓		
Aggressive peck (forceful pecks, usually followed by withdrawal of recipient)	✓				
Litter directed (pecking or scratching at litter)	✓				
Standing only	✓		✓		
Sitting only	✓		✓		
Other (defined separately in each experiment)	✓	✓	✓	✓	✓

[1]At the breast, wings, flanks, back, tail, preen gland and unidentified (vision obscured).
[2] Looked for but not observed.
[3](Nørgaard-Nielsen and Vestergaard, 1981; Vestergaard, 1982; Petherick and Duncan, 1989).

2.3.4 *Recording tools and methods*

All continuous recordings (from both remote and direct observation) were carried out using an Atari® Portfolio hand-held computer (model HPC-004, Atari Corporation, USA) loaded with Keybehaviour software (Deag, 1995). This system was also used for instantaneous recording (Experiment 1), apart from the use of check sheets in Experiment 3. A notebook was used to record any key pressing errors. To collect data using this system, a file was written to enable the Atari® to recognise specific key presses as numbered code. Continuous data collected on the Atari® were downloaded to PC, transcribed from numerical to text code using a translation file, edited for errors, re-checked, and compiled into durations and frequencies per behaviour using the Keytime analysis package (Deag, 1993). Data were then transferred into a spreadsheet programme for condensing before being imported into a statistical package. To transcribe behaviour from the continuous recording stage to formatting for statistical analysis took about an equal amount of time as the sampling period. Instantaneous recordings on the Atari® were downloaded to PC and imported into a spreadsheet programme for editing and condensing before being imported into a statistical package. This process took up to one half the duration of the recording.

The details on recording tools and methods for the initial observations described in 2.3.1 are provided in that section. For all other remote observations, a colour CCTV camera (model WV-CP450/G, Panasonic, Japan) with a domestic video cassette recorder (model HR-J455EK, JVC, Germany) connected through a time/date recorder (model TDG2, VCL, supplied by Heriot Video, Edinburgh) were used. The time/date recorder was required as this information was not recorded independently through the video cassette recorder. All data were recorded onto videotapes, either in real time or in time lapse, so that up to 8 h of data were recorded onto any one videotape. In order to ensure that the video camera was set up correctly, a television monitor was connected to the video cassette recorder so that the recording field could be viewed, but this was switched off during recording or was placed out of sight of the birds in order to prevent social facilitation of behaviour.

Apart from that described in section 2.3.1, all other remote observations were done from an overhead position using a flexible camera-mounting arm (Manfrotto®, Italy) attached to a beam approximately 1.5 m above the pen/cage floor. Using video equipment in this manner reduced the amount of preening events assigned to 'unidentified' and attention to the preen gland could be more easily viewed.

2.4 Preen gland gross morphology and histology

2.4.1 Removing, weighing, and measuring glands

Birds were weighed and then killed using pentobarbitone sodium (Euthatal, Rhône Mérieux Ltd, Harlow, Essex) inserted via the wing vein at a dose of 1ml per kg. Once dead, the bird's skin was pulled back from the base of the tail towards the head to reveal the gland just under the skin's surface. Using surgical scissors and forceps, the gland was cut away from connective tissue and was then weighed on a balance to the nearest 0.1 mg. Using callipers, each gland was measured to the nearest 0.1 mm, for length (top of the papilla to base of the two lobes), depth (from the gland's dorsal to ventral surface), and width (span across the two lobes). Glands for histological examination were stored in labelled plastic containers, in formal buffered saline (1:9 formaldehyde:saline with 3.5 g sodium dihydrogen phosphate and 6.5 g disodium hydrogen phosphate per litre solution), or in a freezer at -20° C until GC preparation.

2.4.2 Histology

To microscopically view the area taken up by the secretory capsules, cavities, interlobular septum and papilla (plus isthmus) in the preen gland, glands were fixed in formal saline, dehydrated, and embedded in wax, which infiltrates the tissues. After cutting, sections were stained and mounted for measurement. Histological techniques used are described below:

TISSUE PROCESSING

Preen glands had been stored in formal buffered saline for up to six months. To dehydrate the glands, each was placed into a porous stainless steel cassette with an identifying label. Cassettes were inserted into a carrier basket of a tissue-processing machine (Histokinette type E7326, British American Optical Co. Ltd., Slough). The basket was dipped in a series of nine alcohol, industrial methylated spirit (IMS), Histosol (a clearing agent, Shandon, Runcorn) and wax baths over a period of 18 h. Sequentially, the baths contained 65%, 75%, 85% absolute alcohol w/w, IMS, two of 100% absolute alcohol, two of Histosol, and wax heated to 60° C (melting point 57° C). The samples remained in the final (wax) bath until removal.

TISSUE EMBEDDING

Once the samples had been dehydrated, each preen gland was fixed into a wax block using an embedding centre (Blockmaster III, Raymond A Lamb, London) as follows. The glands in cassettes were placed into a wax bath heated to 60° C while waiting to be embedded. Each gland was removed from the cassette in turn and placed dorsal side down into a metal mould measuring 35 x 23 mm. This meant that when the sample was blocked, the dorsal side would be sectioned first. A porous, labelled plastic cassette was positioned over the gland and wax heated to 65° C was dispensed into the mould through the holes in the plastic cassette, which bound to the wax when cool. The mould was then placed onto a cold stage set at 0° C so that the wax hardened. After at least 0.5 h on the cold stage, the blocked sample attached to the plastic cassette was removed from the mould and stored until sectioned.

CUTTING SECTIONS

The plastic cassette with blocked sample was clamped into a vice on a microtome (Biocut model 1130, Reichert-Jung, Germany), at a slight angle. This was because the lobes of the gland and the papilla lay in a different plane to the surface of the wax block. By angling the wax block, the lobes at their largest surface area would coincide with that that of the papilla (as judged by eye, where no wider lobe or papilla area was visible

beneath the wax surface). The block was trimmed until a full cross-section of the gland (papilla and both lobes) was cut, then the knife edge was repositioned in order to obtain an unused (sharp) portion of the knife. Cutting thickness was set to 10 μm, after experimentation revealed that this was the thinnest cut possible without the preen gland separating away from the wax.

A section was taken approximately every 5 to 10 cuts, blown on to facilitate ribboning (a common problem with cut sections is that they curl up on themselves), collected onto a microscope slide and placed into a paraffin section mounting bath heated to 40° C. If the sample was still curled before placing into the water bath, a few drops of 20 % ethanol w/w were placed along the sample edge and allowed to run under the sample, causing it to flatten. Sections were then collected onto a labelled glass slide. Only completely intact sections were collected, which ranged from three to five sections per gland. Each slide, which held up to three sections from the same gland, was labelled with a glass cutter and placed on a slide drying hotplate set at 40° C for 48 h before staining. Slides were sometimes coated with Mayer's glycerol albumen to facilitate adherence of sections to the slide during staining. Mayer's glycerol albumen consists of 50 ml egg albumen, 40 ml glycerol, and 1 ml sodium salicylate (Gordon and Bradbury, 1982).

TISSUE STAINING

Slides were placed into a stainless steel slide cradle that positioned them vertically on their longest edge. The slides were then placed into a series of baths for the times specified and for the functions described in Table 2.2.

Table 2.2 *Details of staining method for preen gland samples.*

Bath contents	Time	Function
Histosol	1 min	
100 % absolute alcohol	2 min	Removes wax
IMS	1min	
Running water	1 min	Rehydrates to facilitate uptake of stain
Mayer's Haemalum[1]	5 min	Stains nuclear material
Running water	1 min	Rinses off excess stain
Scott's solution[2]	15 sec	Makes stained nuclear material clearer
Running water	5 min	Rinses off excess stain
Eosin (1 % aqueous solution)[3]	15 sec	Stains non-nuclear material
Running water	15 sec	Rinses off excess stain
IMS	10 sec	
100 % absolute alcohol	1 min	
100 % absolute alcohol	1 min	Dehydrates sample
Histosol	30 sec	
Histosol	30 sec	
Histosol	30 sec	

[1,3] Gurr®, supplied by BDH Laboratory Supplies, Poole.
[2] 3.5 g sodium bicarbonate, 20 g magnesium sulphate, 1 l distilled water.

After staining, the slides were wiped dry around the sample and the sample covered with Entellan mounting medium (Merck, BDH Laboratory Supplies, Poole). A cover slip was placed over the top and the slides were left to dry at room temperature for 24 h before being stored ready for analysis.

2.4.3 Visual imager analysis

In order to measure the areas of the zones (capsules, cavities, papilla (including isthmus) and interlobular septum) of each preen gland, stained sections were measured using a microscope, which was connected to a camera and a PC. Each area was then measured using a computer software package.

A video camera (model TK-S350EG, JVC, Japan) was connected to the x2.5 eyepiece of a microscope (model 229449, Olympus, Tokyo) with a x6.3 objective. The camera

was connected to a PC loaded with Optimas 3.01 visual imager software (Bio Scan Inc). The visual field was calibrated using 5 mm of a ruler so that 5 mm = 4.999 mm (accurate to 1 μm). Due to the size of preen glands after 15 weeks of age, the entire section of a gland could not be viewed at once. Thus, after this age, the gland was viewed in 4 parts by the papilla, left lobe, right lobe, and interlobular septum. The staining procedure used stained the capsule areas dark purple, leaving the cavity areas transparent and the papilla and interlobular septum areas mottled grey. By indicating the contrast threshold of the zone to be measured, the visual imager could identify all areas within the visual field that matched that threshold. Thus, the area of each of the four zones was measured for each section (of which there were two to five per gland), which was collated in a spreadsheet, and mean areas per zone and total area per preen gland were calculated.

2.5 Feather lipid quantity

In order to determine the feather lipid concentration (mg of lipid per g feathers), presumably derived from preen gland secretion but also possibly from the skin, feather samples were collected from birds and processed using a cold (condensed) solvent extraction method, the details of which follow.

2.5.1 Feather collection

Feathers were trimmed from the plumage using surgical scissors, cutting as far down the rachis as possible without injuring the bird. Feathers were taken from the breast, back and flank of birds to get a representative sample of the plumage. Wing and tail feathers were excluded because of their size and weight: one primary feather would have occupied much of the sample. Trimmed feathers were placed in resealable plastic bags and either processed immediately or frozen at -20° C until processing.

2.5.2 *Lipid weight*

Approximately one third of a gram of feathers from each body site was weighed to give a total sample weight per bird of 1.00 g ± 0.01. The sample was placed into a labelled cellulose thimble (27 x 80 mm). Each thimble was placed into the extractor portion of a Soxhlet apparatus (Quickfit glassware), which was filled with enough petroleum ether (AnalR, 40 – 60° boiling point, Merck, Leicestershire) to cover the sample, and soaked overnight for 16 h. This extractant was used because it had previously been shown to be effective at eluting lipid from feathers (van Liere, 1992a), and is known to elute waxes (James, 1995), which are the main lipids found in preen glands (Stevens, 1996). Extraction flasks (150 ml) that had been cleaned, rinsed with petroleum ether, dried in an oven and then cooled in a dessicator for 0.5 h were weighed (to four decimal places) and attached to the bottom of the extractors to collect feather lipid. Once flasks had been cleaned, they were always handled with latex gloves in order to prevent any contamination of dirt or grease, which might affect final flask weight.

The following day, a similar quantity of petroleum ether (as used during soaking) per sample was added to partially fill the round bottom flask and was boiled for 8 h using a heating mantle. The vapour condensed on water-cooled coils and collected in the extractors containing feathers which, when full, tripped an air lock and flushed into the round bottom flasks. At the end of 8 h boiling (24 h total extraction), flasks were removed and excess petroleum ether was allowed to evaporate overnight in a fume cupboard.

The next morning, flasks were placed into a fan oven set at 60° C for at least 0.5 h to remove any remaining solvent and then transferred to a dessicator for 0.5 h to cool, after which they were reweighed to obtain total extracted lipid weight. The concentration of lipid (mg per g feathers) could then be calculated. In one study, a more polar extractant (2:1 chloroform:methanol) was used (Experiment 5b) instead of petroleum ether, in an attempt to elute a greater variety (including phospholipids and triglycerides, in addition to waxes), and thus an overall greater quantity, of lipids.

2.6 Lipid composition

Samples of lipid derived from the preen gland and from feathers were analysed by gas chromatography (GC) to compare fatty acid composition, depending on bird age, housing type, feather pecking status, and oil sample source. Samples were prepared for analysis in the method described below. Confirmation of fatty acid identification was carried out using gas chromatography mass spectrophotometery (GC-MS). The details of collection and preparation are as follows:

Lipid was collected by three different methods over the course of the study: 1) squeezing the preen gland in the live bird and drawing up the extruded oil into a capillary tube, 2) dissecting out the whole preen gland from a dead bird (see section 2.4.1), or 3) removing feathers from the back, flank and breast of birds using sharp scissors (as in section 2.5.1). The samples were either prepared immediately, or were frozen at -20° C until the day of preparation. Frozen whole preen glands were defrosted for up to 2 h before the preen oil was collected from them. Up to 10 samples were prepared at one time.

It was necessary to first isolate the preen oil from other non-lipid molecules such as proteins, cell fragments, etc. The oil sample was placed into a B24 (Quickfit) glass tube with 60 ml 2:1 chloroform:methanol for extraction. If the sample was in a capillary tube, this was placed directly into the B24 tube. In the case of the whole gland, the oil was squeezed out through the papilla or through a small incision in the side of each lobe and collected onto forceps that were then rinsed off in the extractant. Feathers were placed into a paper thimble before then being placed into the tube. All types of samples were then washed with 12 ml 0.88 % KCl w/w to remove unwanted water soluble compounds, which was added to the tube, shaken to mix, and left overnight to separate into two layers.

The following day, the upper (aqueous) layer was pipetted off and discarded. The lower chloroform layer was placed in a 100 ml rotary evaporator flask, and the B24 tube rinsed with 2:1 chloroform:methanol (to ensure removal of all lipid), which was also added to the flask. Approximately 1 ml of methanol was added and the entire solution

dried down under vacuum on a rotary evaporator over a water bath heated to 50° C. Once dry, approximately 1 ml of chloroform and 1 ml of methanol were added to the flask, which was dried again on a rotary evaporator. Lipid was redissolved in roughly 2 ml 2:1 chloroform:methanol and the contents of the flask were removed to a glass scintillation vial.

Thin layer chromatography (TLC) was then necessary in order to isolate out the predominant wax fractions to be analysed (preen oil in chickens is primarily composed of diester waxes; Haahti and Fales, 1967; Kolattukudy and Sawaya, 1974). Clean glass TLC plates were coated in 0.25 mm of silica gel (Kieselgel 60, Merck, Leicestershire) solution (22.5 g silica gel with 50 ml distilled water made 5 plates) using a plate spreader. Plates were then allowed to dry for at least 1.5 h in an oven (set at 100° C), then removed from the oven and left to cool just prior to use.

The solution in the vial was dried on a heating block at 55° C in the presence of oxygen-free nitrogen (to prevent oxidation). For samples that had been collected in capillary tubes, the sample amount was so small that it was dissolved in 150 µl chloroform, which was used in its entirety for TLC. In the case of oil taken direct from the whole gland, or from feathers, there was too much sample present to use the entire amount. (A TLC plate with a silica gel layer of thickness 0.25 mm can hold up to 20 mg of sample; Henderson and Tocher, 1992). Therefore, in those instances, gravimetric weights were determined: a clean scintillation vial was weighed to four decimal places, while 500 µl chloroform was added to the dried sample. Once the lipid had dissolved, 100 µl of this was added to the weighed vial, dried in the presence of nitrogen, and the vial was re-weighed. This gave lipid concentration (mg per 100 µl solution). From this calculation, the appropriate amount of solution (µl) could be spotted onto the plate with a known amount (mg) of lipid in it, so that the 20 mg limit per TLC plate would not be exceeded.

TLC plates were then spotted with the lipid sample (up to three samples per plate) approximately 1.5 cm from the bottom of the plate and 1 cm in from the plate edge. Each sample was separated on the plate by a pencil-drawn line. Spotted plates were developed in a solution of 80:40:1 hexane:diethyl ether:formic acid in a glass

developing tank fitted with a lid. Two pieces of absorbent paper were draped into the tank to encourage the solution to saturate the atmosphere. The plates (up to two per tank) were left for 30 – 55 min to allow the solvent to migrate up the plate. Plates were removed from the tank when the solvent front reached approximately 2 cm from the top of the plate, and then left in a fume cupboard for a few minutes until the solvent had evaporated. Once dry, plates were sprayed with dichlorofluorescein and viewed using a UV light. This revealed several bands of lipids, and the uppermost band, which should contain the wax esters (Henderson and Tocher, 1992), was scraped off for each sample and placed into a centrifuge tube.

To remove lipid sample from the silica gel, approximately 2 ml of diethyl ether were added to the sample, mixed, and centrifuged at 630 *g* for two minutes. The supernatant was poured off into a rotary evaporator flask, and the diethyl ether mix and centrifuge step was repeated.

Each wax ester at this stage was comprised of a long-chain alcohol and a fatty acid joined by an ester (-COO) bond (two ester bonds with diesters). To isolate the fatty acid for GC analysis, methylation was carried out to split the bond, resulting in a fatty acid with a methanol group replacing the alcohol portion, and a reformed long chain alcohol. (Methanol (CH_3OH) dontes the -CH_3 group to the fatty acid portion, and the -OH group to the alcohol portion.) To do this, the sample was evaporated to dryness on a rotary evaporator. To each sample, 4 ml methylating agent (20:10:1 methanol:toluene:sulphuric acid) was added and refluxed for 30 – 120 min. (Reflux consisted of heating the solution on a heating mantle and condensing the vapour on a water-cooled coil). Following refluxation, the solution was allowed to cool for approximately 15 min and then 10 ml of both distilled water and hexane were added to the solution. This was mixed vigorously and poured into a B19 tube and allowed to separate into two layers. The top hexane (methylated fatty acids) layer was pipetted off into another B19 tube that contained drying agent (4:1 sodium sulphate:sodium hydrogen carbonate) in order to remove last traces of water. After 30 min, the hexane was removed into a smaller, B14 tube and dried on a heating block at 55° C in the

presence of nitrogen. Once dry, the tube was rinsed with a few drops of hexane and this solution was placed into a 1.1 ml autosampler vial (Chromocal) until analysis.

Once prepared for GC, the samples were stored at 4° C until processing. They were then analysed using a gas chromatograph (Chrompack, model CP 9002, Netherlands) with a silica column 60 m in length with an inside diameter of 0.22 mm. The stationary phase lining the column was BPX-70 (SGE, Australia). Approximately 1.5 µl of sample was auto-injected into a splitter injector, which took a proportion of the sample at a ratio of 1:40 (this is to prevent overloading the column). The sample was vaporised at 250° C and then carried into the coiled column using helium as the transport gas. The column, which sits in an oven, was set at an initial temperature of 70° C, at which it was held for 4 min on injection of the sample. It then increased by 10° C min^{-1} until it reached 180° C (this is to separate out short chain methylated fatty acids up to C8:0). The temperature increased further by 2° C min^{-1} until it reached 220° C (to separate out long chain methylated fatty acids up to C24:0) and was then held at 220° C for 5 min. Finally, the coil was heated to 240° C and maintained for 7 min, in order to flush through any remaining molecules before cooling to 70° C in preparation for injection of the next sample.

At the end of the column was a flame ionisation detector (FID), which consisted of a flame burning in an air and hydrogen environment between two electrodes. As each methylated fatty acid was burned in the flame, the electrodes detected a) the time at which the acid came through the column (retention time), thus identifying the acid by comparing its retention time to that of a known standard, and b) the quantity of carbon ions generated when the fatty acid was burnt (measured in millivolts), which determines the quantity of that fatty acid present in the sample.

The output from the FID was interpreted by a data handling software package (EZ-Chrom, Scientific Software Inc, USA) on a PC. This generated a table of retention times and the proportion of each fatty acid of the total sample, plus a chromatogram

(graph of chromatography output). In this way, the methylated fatty acids from preen oil were identified and quantified as a proportion of the total sample injected.

2.7 Statistical analysis

To check for normality, data were plotted in histograms (frequency distributions) or residual vs fitted values graphs to examine for constant variance. The duration of total time spent preening and times spent preening each body site were often positively (left skewed) distributed when graphed, or there were unequal variances between treatments. Where this was the case, date were transformed to give approximately normal distributions and equal variances, and then analysed using parametric tests. Methods of transformation included angular (arcsine root), arcsine, empirical logit, +1 log, and natural log (ln). Figures listed are original figure means ± standard error of the mean, or standard error of the difference, for clarity. On one occasion (Experiment 5b), data could not be normalised and in that case, the Mann-Whitney U-test was used. All other data, such as body weights, mandible lengths, and feather scores, were analysed by t-test, ANOVA, REML, correlation, or regression, after being observed to follow normal distributions. Details on analysis of data sets are set out in each chapter. All analyses were carried out using Minitab Version 12 or Genstat 5 Release 4.1.

2.7.1 Bout analysis

Since preening has not yet been studied in detail in the laying hen, an attempt was made to determine what constituted a preen bout. Hall (2001) used number of preening bouts, and mean bout length, to describe broiler behaviour at different stocking densities, but did not define how bouts were calculated, apart from 'the rate at which preening was initiated'. LeFebvre and Joly (1982) examined temporal organisation of preening in kestrels, however they assumed in their methods that the bout interval was 2 min, and did not explain how this figure was reached. Here, a bout is defined as a group of behavioural events (preening) separated by a calculated minimum interbout length known as the bout criterion interval (Slater, 1974a). All events of preening that are separated by gaps in preening behaviour shorter than the bout criterion are thus within a

bout, and those that are separated by gaps equal to or longer than the bout criterion are in different bouts.

A popular method for bout analysis has been log survivorship, in which the log of cumulative frequencies of gap lengths are plotted (on the y-axis) against gap lengths (x-axis) (Slater, 1974a; Slater, 1974b; Slater and Lester, 1982). Where the graph of the line 'breaks' (the slope changes, thus looking like two lines joined together), the corresponding x-coordinate of gap length indicates the bout criterion interval. However, where the line breaks is determined by eye, and is therefore not objective. More recently, Sibly *et al* (1990) designed a non-linear regression analysis method called log frequency. This plots natural log (ln) frequency of gaps against gap durations, and then uses non-linear regression to objectively determine where the break lies between the two lines (2-process model). Another method devised by Berdoy (1993) examined the use of a 3-process model, in which a behaviour could be divided not only into bouts (using a calculated bout criterion interval), but bouts could be grouped into clusters (using a calculated cluster criterion interval). The plot of data that fits this process would resemble 3 lines joined together. The following analysis on data from Experiment 5a employs the 2-process log frequency method described by Sibly *et al* (1990) using formulae from Slater and Lester (1982). This method was chosen over the 3-process model because it was simpler to apply and interpret.

All gaps between preening, and comfort movements where they fell within 60 sec of a preen movement, were extracted from data on 16 hens video recorded for one light cycle (16 h) on either wire or litter floors (see Experiment 5a for full experimental details). Due to the limitations of the recording device, gaps of less than 1 sec were not recorded. This yielded a total of 9909 gaps of varying lengths (in sec) from all birds.

All gaps were listed in ascending order by gap length (sec) and number of occurrences (f). The number of occurrences of a given gap was divided by the interval (w) between that gap and the next gap on the ascending list to give the frequency of each gap per time unit (z):

$$z = \frac{f}{w}$$

(Equation 2.1)

For example, in the data used here, there were 8 occurrences of gap length 30 sec (gap_{30}). The next gap length that occurred was 31 sec (gap_{31}). Therefore, $f_{30} = 8$, $w_{30} = 1$ sec, thus $z_{30} = 8/1 = 8$.

The frequency (z) was ln transformed (in this example, ln 8 = 2.08) and plotted against the midpoint between the two gaps (here, 30.5). Ln transformation is necessary to equalise the variances at different gap duration values, in order to use non-linear regression analysis.

This process was repeated for all gap lengths and number of occurrences from gap_1 to gap_{445}. The graph of ln frequency against the midpoint of gap length beyond 445 sec became so random, and the number of occurrences of longer gaps beyond this were so infrequent, that they were not plotted.

A plot of the gap length midpoints by ln frequency of the data up to gap length 100 sec is shown in Figure 2.2, as this shows enough data to reveal where the estimated change in slope occurred. (This is derived from total data from 16 hens). Where the line of ln frequency of gap lengths has the steepest slope indicates within bout gaps (fast process), and where the slope decreases indicates gaps between bouts (slow process).

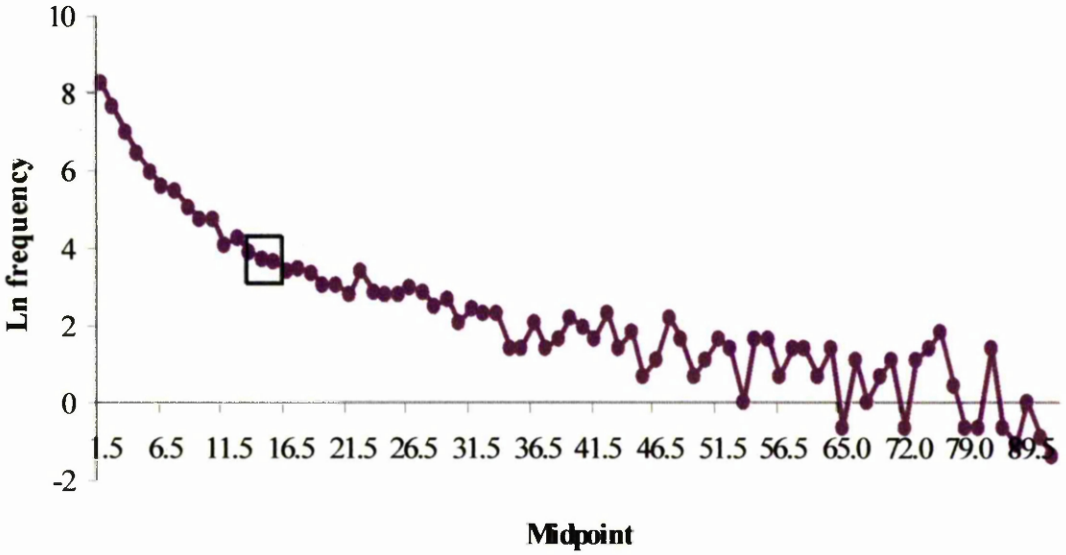


Figure 2.2 *Midpoint of gap length (in sec) against ln frequency. The selected area is where the end of the fast process (x, y co-ordinates 14.5, 3.71) and the beginning of the slow process (15.5, 3.64) were estimated (by eye).*

The initial estimates of the end of the fast process (x,y co-ordinates) were (14.5, 3.71) and the beginning of the slow process were (15.5, 3.64), as judged by eye (these co-ordinates are indicated by the black box in Figure 2.2). These initial estimates of the parameters must be made for non-linear regression analysis to calculate the true parameters (x, y co-ordinates) of where the fast process ends and the slow process begins, but how accurate the initial estimates are do not affect the final bout criterion: different estimates result in the same bout criterion (Berdoy, 1993). All parameters that described the fast process line and the slow process line were analysed by regression to estimate y-intercept and slope of each line.

For a line plotted using ln frequency distribution, the formula describing a line where ($\ln N\lambda$) describes the y-intercept and ($-\lambda$) describes the slope is:

$$y = \ln N\lambda - \lambda t$$

(Equation 2.2)

In the case of two processes, there are two lines and therefore:

λ_f = mean number of gaps per second within bouts (fast process)

N_f = total number of gaps within bouts (fast process)

λ_s = mean number of gaps per second between bouts (slow process)

N_s = number of gaps between bouts (slow process)

For the fast process line, the initial estimates using linear regression were slope (λ_f) = 0.3313 and y-intercept ($\ln N_f\lambda_f$) = 8.112. In order to calculate N_f:

$$\ln N_f\lambda_f = 8.112, \text{ then } N_f = \frac{e^{8.112}}{0.3313} = 10064$$

(Equation 2.3)

Likewise, for the slow process line, regression analysis gave slope (λ_s) = 0.008766 and y-intercept ($\ln N_s\lambda_s$) = 1.669. Using the same logic as in (Equation 2.3, N_s = 605.3.

Once these estimates were calculated for the two lines, the x and y co-ordinates of both lines were analysed by non-linear regression analysis, using the estimates as shown above, which gave the figures:

λ_f = 0.132

N_f = 4128

$\lambda_s = 0.005$

$N_s = 384.5$

($R^2 = 84.3$)

From these results, there were two methods of determining the bout criterion interval, one which ultimately minimised the total time misassigned (t_{c1}), and the other which minimised the number of intervals misassigned (t_{c2}), to the wrong (fast or slow) process. These formulae are:

$$t_{c1} = \frac{1}{\lambda_f - \lambda_s} \ln \frac{N_f}{N_s}$$

(Equation 2.4)

$$t_{c2} = \frac{1}{\lambda_f - \lambda_s} \ln \frac{N_f \lambda_f}{N_s \lambda_s}$$

(Equation 2.5)

Using the parameters given by non-linear regression analysis, t_{c1} = 18.7 sec and t_{c2} = 44.5 sec. For either t_c, Slater and Lester (1982) demonstrated that the number of intervals misassigned was:

$$N_f e^{-\lambda_f t_c} + N_s(1 - e^{-\lambda_s t_c})$$

(Equation 2.6)

Where t_{c1} = 107.3 sec, then the number of intervals misassigned was 384.0.

Where t_{c2} = 44.5 sec, then the number of intervals misassigned was 88.3.

The least number of intervals are misassigned using t_{c2}, therefore this gives the most accurate bout criterion, which was rounded to 45 sec. Thus, 45 sec was the minimum inter-preening bout interval. The calculated bout criterion of 45 sec differed largely from the initial estimate of approximately 15 sec, as judged by eye in Figure 2.2. The break in the slope was not easily seen, due to the randomness of the line, and this resulted in a poor estimate.

Chapter 3

Experiment 1 - Ontogeny of Preening in Layer Pullets

3.1 Introduction

Beak trimming is a technique widely used in the UK egg industry to control feather pecking and cannibalism, common causes of feather loss, injury and mortality, and hence also economic loss for producers. Beak trimming (or beak tipping) is a contentious issue due to the potential acute pain the procedure causes in cutting through tissue innervated with nerves (beak trimming is not required to be carried out under anaesthesia; MAFF, 1996). Gentle (1986b) showed that beak trimming one third of the upper and lower mandibles in 5-week old chicks resulted in scar tissue formation, into which regenerating nerve fibres could not grow. By 20 days post-amputation, neuromas (bundles of regenerated nerves that show spontaneous activity) had formed in the tissue adjacent to the scar (Gentle, 1986b). Beak trimming has been shown to cause pain, as indicated by reduced environmental pecking in adult hens up to 6 weeks after trimming, which suggests guarding behaviour of a painful body site (Gentle *et al*, 1990). Beak trimming in adult hens was shown to lead to reduced food intake, possibly due to pain, reduced feeding motivation, or inaccuracy in aim while pecking at food (Gentle *et al*, 1982).

Most previous studies on the effects of beak trimming have not looked at trimming of laying hens in a commercial context, or have not followed the effects on behaviour. In the UK egg industry, beak trimming is usually carried out at 7 – 10 days of age by a hot blade debeaker (Barron Ltd, Daylay Foods, Deans Farm, Glenrath Farms, Hubbard ISA, Joice & Hill Poultry Ltd, all pers. com.). Work by Duncan *et al* (1989) and by Gentle *et al* (1990) used 16 week-old hens and only followed the effects of trimming on behaviour for up to 6 weeks. In another study by Gentle *et al* (1982), 80 week-old hens were used and only feeding behaviour was observed, omitting other oral behaviours that may be affected, such as drinking, preening or pecking at the environment. Turkey poults that were beak trimmed at 6 or 21 days of age using a hot blade debeaker showed

no evidence of neuroma formation 42 days later (Gentle *et al*, 1995), but no observations of behaviour took place. Similar work on turkey poults done by Grigor *et al* (1995) followed the effects of beak trimming on behaviour, body weight gain, feather pecking damage and beak regrowth (as in this study), but only to 12 weeks of age. Blokhuis and van der Haar (1989) examined the effects of beak trimming on behaviour and feather pecking damage during rearing and laying periods, however beak trimming was carried out when the birds were 45 days old. The most relevant study to date was that by Gentle *et al* (1997) in which ISA Brown chicks were beak trimmed or sham-operated by hot or cold cut at 1 or 10 days old, and then observed for behaviour, body weight gain and feather damage scores. However, behaviour data were collected for only 6 weeks post-trim, as opposed to 18 weeks post-trim in the present study.

The purpose of this study (Experiment 1) was to look at the effects of standard commercial beak trimming practice on preening and other behaviours over 20 weeks in layer pullets. Feather damage scores and mandible lengths were also recorded to assess the effects of beak trimming on plumage condition and beak regrowth.

3.2 Materials and Methods

Ninety-six ISA Brown chicks were housed from day old to 20 weeks of age in 12 pens with a floor area of 2.50 m^2 in a windowless room, with 8 birds in each pen (3.2 birds/m^2). Pen floors were covered in litter (woodshavings). There was one bell drinker and feeder provided per pen, containing *ad libitum* supplies of water and food. The diet was changed from (standard) starter mash to grower mash at 7 weeks of age, and from grower to layer mash at 16 weeks of age. The photoperiod was changed according to the breeding company's guidelines (22 h reduced to 16 h over the first week, then reduced by 1 h weekly to 10h, which was maintained from 7 to 14 weeks of age, then increasing to 13.5 h from 14 to 20 weeks of age; Hubbard ISA, 1998). Mean light intensity at bird head height was 14 lux and mean ambient temperature was 18° C.

3.2.1 *Beak Trimming and Mandible Length*

When the chicks were 8 days of age, all the chicks in 6 pens chosen at random for the beak trimming (BT) treatment were beak trimmed using a hot blade Lyon Debeaker® (Lyon Electric Company, Inc). Of the 3 diameters on the debeaker, which affected how much of the beak was removed in cutting and cauterisation, the second largest (approximately 4 mm in diameter) was chosen. This resulted in about one quarter of both mandibles being removed (the most severe beak trimming treatment permitted in the UK is one third of the upper and lower mandibles; MAFF, 1996). The remaining birds in the other 6 pens were handled as for beak trimming but were left with their beaks intact (NBT). In the final week of the study, when the birds were 20 weeks old, every bird's upper and lower mandibles were measured, using the method described in Chapter 2 section 2.2.3. This was to assess how beak trimming chicks affected beak regrowth of adult birds.

3.2.2 *Body Weight*

To monitor growth rates and the effect of beak trimming, birds were weighed on arrival at day-old, then once a week to 4 weeks of age, and at 8, 12, 16 and 20 weeks of age.

3.2.3 *Behaviour Observations*

Birds in each pen were observed systematically 4 times a week for 20 weeks, from the week of arrival (0 weeks of age) to 19 weeks of age. Observations were carried out twice in the morning (between 0800 and 1300 h) and twice in the afternoon (between 1400 and 1800 h), with pens being observed in a different random order on each occasion. Each pen was observed during a 15-min session, when the behaviour of each bird (identified from a uniquely coloured leg ring) was recorded every minute from a single 'on the beep' observation (Martin and Bateson, 1993), according to one of 13 mutually exclusive categories. These were standing, sitting, walking, feeder or drinker directed, preening while standing or sitting, litter directed (both pecking and scratching), dustbathing, comfort behaviour, spot pecking, and non aggressive or aggressive pecking. All wing flapping, body shaking, head scratching, stretching, billwiping and

pecking at the legs or feet were recorded as one category (comfort activities). No association with preening could be inferred due to the recording technique. From the recordings, mean proportions were calculated out of time spent in the various activities.

All birds were assessed for pecking damage to their plumage at 5, 10, 15 and 20 weeks of age, using the method described in Chapter 2.

Mandible lengths, body weights, and plumage damage scores were calculated for pen means ± standard error of the difference between beak treatments, and then analysed using t-test. The behavioural data were transformed by arcsine transformation and were then compared using two-way ANOVA to measure the significance of effects of age, beak treatment and their interaction. A separate analysis was done for each of the 13 activities, but preening while standing and preening while sitting were also analysed in more detail for times spent in attention directed at the breast, wings, back, flanks, tail or preen gland. The mean values ± standard error presented in Table 3.2 and Table 3.3 are in the observed scale.

3.3 Results

Of the 96 birds in the experiment, one was culled at 19 weeks of age and one at 20 weeks of age, both due to pecking damage to the head. Both of these birds came from non-beak trimmed pens. There were no significant differences ($P > 0.05$, by t-test) in mean body weights between beak treatments at any age, including the week immediately following beak trimming (Table 3.1). Birds came into lay at 17 weeks of age.

Table 3.1 *Mean body weights (g) of pens of birds at different ages, according to beak treatment*[1].

Age (weeks)	0	1	22	3	4	8	12	16	20
BT	35	72	133	219	325	821	1283	1537	1774
NBT	35	72	133	215	320	802	1247	1497	1742
SED[3]	1	2	3	4	6	13	17	23	38
t-value	0.84	0.19	0.09	0.86	0.81	1.40	2.06	1.76	0.92

[1]BT = beak trimmed (n =6); NBT = non-beak trimmed (n =6).
[2] One week after beak trimming.
[3]SED = standard error of the difference between means, with 10 degrees of freedom.

From the behavioural observations, the main activities of these birds were standing, sitting, walking, feeder directed, preening (mainly while standing), and litter directed (Table 3.2). Little time was spent in drinker directed, dustbathing, comfort behaviours, spot pecking or bird to bird pecking of either type. All observed behaviours were significantly affected by bird age (Figure 3.1 and Figure 3.2) apart from dustbathing (Appendix B). Preening and dustbathing were seen on the first day of observation, when the chicks were 3 days of age. Preening while sitting declined with age whereas preening while standing increased. Preening overall (in stand and sit) increased with bird age. There was virtually no aggressive pecking until the last 2 weeks (18/19 weeks of age), in which aggressive pecking in both BT and NBT birds was significantly higher (0.73 %) than in all previous 2-week periods ($P < 0.001$, by two-way ANOVA). Sitting and comfort behaviour declined steadily with age. Beak trimming had a significant effect on litter directed behaviour, where NBT birds showed more activity than did BT birds. There was no affect of interaction of age x beak treatment on any of the behaviours observed.

Table 3.2 *Overall mean ± standard error and significance of effects of bird age (from 0 to 19 weeks, 10 x 2 week blocks) and beak treatment (BT vs NBT), by two-way ANOVA (on arcsine transformed data, figures presented here are original percent figures) on behaviour*[1].

Behaviour	Overall mean (%) ± SE	Age	Beak treatment	Mean (%) BT	Mean (%) NBT
Standing	26.4 ± 0.6	***	-		
Sitting	10.4 ± 0.9	***	-		
Walking	15.0 ± 0.3	***	-		
Feeder directed	15.4 ± 0.5	***	-		
Drinker directed	1.9 ± 0.1	***	-		
Preening (stand)	8.5 ± 0.5	***	-		
Preening (sit)	2.3 ± 0.2	***	-		
Litter directed	14.0 ± 0.5	***	*	12.6	15.4
Dustbathing	0.6 ± 0.1	-	-		
Comfort	3.2 ± 0.1	***	-		
Spot pecking	0.9 ± 0.1	***	-		
Non-aggressive pecking	1.2 ± 0.1	***	-		
Aggressive pecking	0.1 ± 0.0	***	-		

[1]Significance levels: *** = $P < 0.001$; * = $P \leq 0.05$; - = $P > 0.05$.

Figure 3.1 *Mean proportion (%) of time spent in frequent behaviours that varied significantly with age (with standard error bars).*

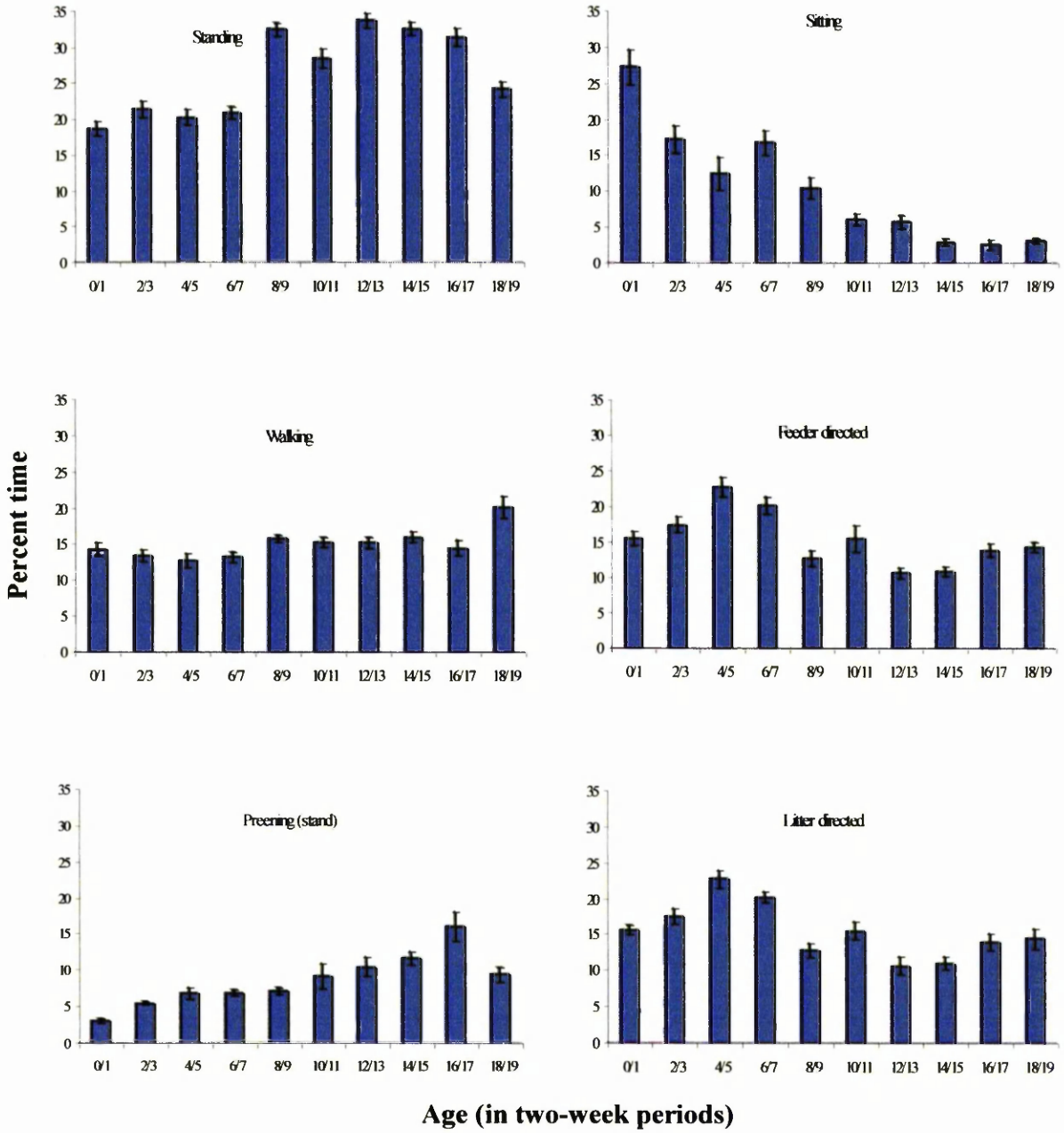
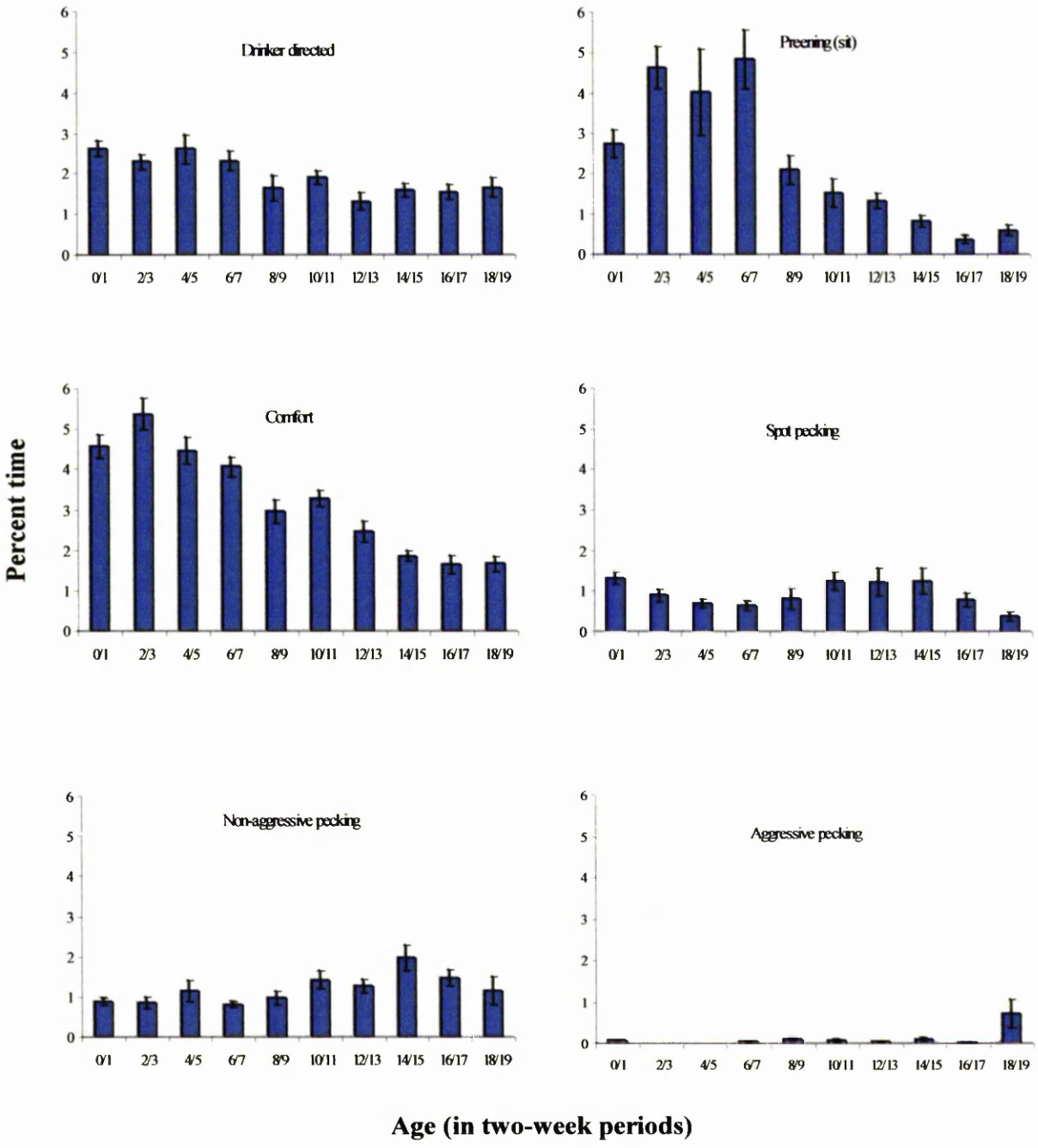


Figure 3.2 *Mean proportion (%) of time spent in less frequently seen behaviours that varied significantly with age (with standard error bars).*



Of the total proportions of time spent preening, attention directed at the wings accounted for most time, whereas that directed at the preen gland accounted for least time, both while standing and sitting (Table 3.3). BT birds directed significantly more attention than did NBT birds at the preen gland, while standing and sitting, and at the back while sitting. Attention to the preen gland was first seen at age 2/3 weeks. Preen gland use was highest at age 6/7 weeks while sitting (0.03 %) and at age 8/9 weeks while standing (0.14 %).

Table 3.3 *Overall mean ± standard error, with significance of effects of bird age (from 0 to 19 weeks, 10 x 2 week blocks) and beak treatment (BT vs NBT), by two-way ANOVA (on arcsine transformed data, figures presented here are original percent figures) on detailed preening behaviour*[1].

Behaviour	Overall mean (%) ± SE	Age	Beak treatment	Mean (%) BT	Mean (%) NBT
Preening (stand):					
Breast	2.2 ± 0.1	***	-		
Wings	3.3 ± 0.2	***	-		
Back	1.4 ± 0.1	***	-		
Flanks	1.2 ± 0.1	***	-		
Tail	0.4 ± 0.0	***	-		
Preen gland	0.1 ± 0.0	**	*	0.09	0.04
Preening (sit):					
Breast	0.4 ± 0.0	***	-		
Wings	1.1 ± 0.1	***	-		
Back	0.6 ± 0.1	***	*	0.71	0.48
Flanks	0.1 ± 0.0	***	-		
Tail	0.1 ± 0.0	***	-		
Preen gland	0.0 ± 0.0	*	*	0.01	0.00

[1]Significance levels: *** = $P < 0.001$; * = $P \leq 0.05$; - = $P > 0.05$.

When comparing the mandible lengths of BT and NBT birds at 20 weeks of age, the upper mandible, but not the lower one, was significantly shorter in BT birds than in NBT birds (Table 3.4). NBT and BT birds also differed significantly in the difference between upper and lower mandible lengths.

Table 3.4 *Mean beak measurements of BT (n=6) and NBT (n=6) pens of birds taken at 20 weeks of age, and their significance, by t-test.*

Measurement (in mm)	NBT	BT	SED[1]	t-value	P value
Upper mandible length	33.9	30.8	0.25	-10.19	< 0.001
Lower mandible length	32.0	32.4	0.37	0.75	> 0.05
Difference between upper and lower mandible lengths	1.9	-1.6	0.25	-8.08	< 0.001

[1]SED = As in Table 3.1.

There was no significant difference ($P > 0.05$, by t-test) in mean plumage damage score between BT and NBT birds at any age measured. Mean scores (and SED, with 10 degrees of freedom) of BT and NBT, respectively, were 0.00 and 0.00 (0.00) at 5 weeks of age, 0.00 and 0.02 (0.02) at 10 weeks of age, 0.04 and 0.00 (0.04) at 15 weeks of age, and 0.06 and 0.19 (0.20) at 20 weeks of age.

3.4 Discussion

The only birds that needed to be culled during this trial came from pens in which birds had not been beak trimmed. Although there was very little aggressive pecking overall, it was increasing as the birds matured, and the severity of pecking in two NBT pens made culling of two birds necessary. The lack of aggressive behaviour and plumage damage in these birds may be a consequence of the small, stable groups (8 birds per pen) and early sexual maturity at which the observations terminated. Feather pecking and cannibalism are partly influenced by hormonal factors that can result in increases in these behaviours around the onset of lay (MAFF, 1981; Keeling, 1995). Hughes (1973) showed that artificial implants of oestrogen/progesterone significantly advanced the onset of feather pecking (compared to controls) in sexually maturing pullets. Plumage damage was increasing overall at the end of the study, at 20 weeks of age, when damage was higher (but not significantly so) with the NBT birds than the BT birds (0.19 vs 0.06), as was expected based on previous work (Hughes and Michie, 1982).

Beak trimming did not significantly affect body weight at any age, including one week after trimming took place (at 2 weeks of age), in contrast to previous studies (Blokhuis *et al*, 1987; Gentle *et al*, 1997; Hadorn *et al*, 1998). Blokhuis *et al* (1987) beak trimmed approximately one third of the upper and lower mandibles of pullets at 45 days of age, resulting in a significantly lower mean body weight compared to controls at 17 weeks of age. Gentle *et al* (1997) found that beak trimming significantly depressed body weight gain compared to controls in the first 1 to 2 weeks after trimming, with both hot cut trimming at 10 days of age and cold cut trimming at 1 and 10 days of age. In the first two weeks after beak trimming at day-old, Hadorn *et al* (1998) noticed a slight reduction in body weight of trimmed birds compared to controls. The lack of beak trimming effects on body weight in this study may be due to the small amount of beak removed during trimming (up to one quarter) compared to one third (Grigor *et al*, 1995; Gentle *et al*, 1997) or one half (Duncan *et al*, 1989) in other studies.

There were few effects of beak trimming on the oral behaviours in these birds. Previous work has indicated that there is little or no neuroma formation if beak trimming is done in young birds (Gentle *et al*, 1995). Duncan *et al* (1989) found that older birds subjected to beak trimming reduced the time spent feeding and preening compared to pre-treatment values. In this study however, BT birds did show significantly less time in litter directed behaviour than NBT birds, but this included scratching as well as pecking at litter. This is similar to the findings of Blokhuis and van der Haar (1989), who observed that birds beak trimmed at 45 days of age spent significantly less time ground pecking and less time ground scratching (but not significantly so) up to 17 weeks of age, compared to non-beak trimmed birds. The fact that no other oral behaviours were significantly affected by beak trimming suggests that the operation did not cause chronic discomfort in these birds. Even though beak trimming early in life may not result in chronic pain associated with neuroma formation behind scar tissue (Gentle *et al*, 1997), it may still cause reduced sensory perception due to removal of touch receptors in the beak tip.

Chicks showed preening and dustbathing behaviour on the first day of observation at 3 days of age, which is similar to Kruijt (1964), who observed red junglefowl chicks first

showed preening and dustbathing movements at 1 and 2 days of age, respectively. More preening occurred while standing than while sitting. Preening while standing increased and preening while sitting decreased with age, reflecting the increase and decrease in times spent standing and sitting with age. Preening increased overall with bird age, which is similar to Dawson and Siegel's (1967) findings, in which preening increased linearly over 0 to 10 weeks of age in White Rock chicks. Lee and Craig (1990) also found that preening in layer pullets increased significantly from 4 to 16 weeks of age. Preening behaviour overall was not affected by beak treatment, even in the first 2 weeks after beak trimming, as shown by a lack of age x beak treatment interaction. This is in agreement with studies by Lee and Craig (1990) and Kuo *et al* (1991) who found that chicks with half or one quarter of their beaks trimmed at 24 days of age did not differ in time spent preening up to 16 weeks of age compared to controls. Likewise, Lee and Craig (1991) found that layer pullets beak trimmed at 4 weeks of age showed no differences in time spent preening at 24 weeks of age compared to intact birds. Van Liere (1995) also found that beak trimming layer pullets at 6 weeks of age did not affect time spent preening at 42 weeks of age compared to intact birds. These contrast with Grigor *et al's* (1995) study, in which turkeys that were beak trimmed at 1, 6, and 21 days of age showed significantly less time preening over a 12 week observation period post-trim than did controls, and also with Gentle *et al's* (1997) study, in which there was less preening in the first week after beak trimming in birds beak trimmed at 1 and 10 days of age compared to controls.

In detailed analysis of preening in the present study, BT birds directed significantly more attention at the preen gland than did NBT birds, whether standing or sitting. This suggests that BT birds took longer to collect preen oil from their preen glands, presumably because of the removal of their sensitive beak tips. BT birds also spent significantly more time preening at the back than did NBT birds, but only while sitting. Overall, most time was spent preening the wings, possibly due to the evolutionary importance of maintaining their condition for escape behaviour. Least time was spent at the preen gland, probably due to the infrequency of oiling behaviour during preening (van Liere *et al*, 1991). Preen gland use was first seen at age 2/3 weeks, which is similar to Williams and Strungis (1979) who first observed oiling behaviour at 28 days

of age in White Rock chicks. (Here, at the end of 3 weeks of age, chicks were 27-days old). This contrasts to Kruijt's (1964) observations in which red junglefowl chicks first showed head-rubbing with rotation at the rump (presumably to collect preen oil) at 11 days of age. However, in this study, dustbathing behaviour was evident from the first few days of life, suggesting that it is not solely the presence of preen oil on the plumage that stimulates dustbathing. This is supported by the findings of Nørgaard-Nielsen and Vestergaard (1981) who found that in fact preen gland extirpated hens dustbathed sooner and for longer compared to hens that had not been operated on, after 27 or 75 h of dustbathe substrate deprivation. Williams and Strungis (1979) also noticed that dustbathing (first seen at 6 days of age) began before oiling (28 days of age). Dustbathing is most likely internally, as well as externally, motivated, and it may be that the presence of sebaceous secretions from the skin (Bolliger and Varga, 1961; Ishida *et al*, 1973) contribute to dustbathing motivation.

The method of behavioural data collection used here (scan sampling with instantaneous recording) is not as accurate as continuous recording in which all occurrences of the behaviours of interest are recorded for their true durations. Here, preening represented a total of 10.8 % (preening while standing plus preening while sitting) of the time observed, which is similar to levels shown in another study in which the 'on the beep' method was used (9.1 %; Savory and Mann, 1997).

Beak trimming is still widely used in the laying hen industry due to its effectiveness at reducing feather pecking, cannibalism and associated mortality, as supported by previous studies (Appleby *et al*, 1988; Grigor *et al*, 1995; Bell and Adams, 1998). The results of this study support earlier findings that beak trimming at a young age prevents neuroma formation (Gentle *et al*, 1995), as indicated by the lack of change in most beak related behaviours. The altered dimensions of the tip of the beak may affect the accuracy of aiming at precise areas, such as at the preen gland, during preening, but overall there were only a few effects of beak treatment on preening and other behaviours, and body weight was not significantly affected. Upper mandible lengths and the difference between upper and lower mandible lengths differed between BT and NBT birds at 20 weeks of age (19 weeks after beak trimming), indicating that beak

trimming at a young age (8 days) can have lasting effects on beak morphology. Furthermore, there was a suggestion that plumage damage scores were increasing more in NBT birds when the study ended at 20 weeks of age.

Chapter 4

External Influences

4.1 Introduction

Previous studies of fowls indicate that preening behaviour can be influenced by the birds' environment. Increases in time spent preening have occurred with changes in food form (pellets vs mash; Savory and Hetherington, 1997), and during visual access to conspecifics (Nicol, 1989). Preening can occur in apparently inappropriate contexts, during arousal; for example during aggressive encounters in red junglefowl (Kruijt, 1964) and during pre-laying behaviour in laying hens in the absence of nest-building material (Mills and Wood-Gush, 1985). The purpose of the experiments in this chapter was to examine how the birds' environment affected times spent preening, at different body sites, and relationships among preening, time of day, dustbathing and oral behaviours.

Initially in this study, a distinction was sought between what constituted functional from non-functional (displacement) preening. Displacement preening, in particular, which is described as being more frantic and rushed in appearance than functional preening (van Iersel and Bol, 1958), has been demonstrated to occur in relation to food frustration (Duncan and Wood-Gush, 1972a; Duncan and Wood-Gush, 1972b). This was indicated by a greater number of preen movements (time from bill contacting feathers until head was raised) that were shorter in duration compared to during the non-frustrating period (Duncan and Wood-Gush, 1972a), and a higher proportion of time spent preening at near (breast, belly, shoulder, outside wing) as opposed to far (inside wing, back, tail, vent, preen gland) body sites (Duncan and Wood-Gush, 1972a). Other studies have suggested that increases in total time spent preening by food-restricted broiler breeders compared to *ad libitum* fed birds is indicative of frustration (Kostal *et al*, 1992; Hocking *et al*, 1996).

Previous studies on group-housed hens suggest that the performance of certain behaviours, such as feeding or preening, may attract feather pecking, and that feather

peckers tended to be more active (Keeling, 1995; Keeling and Jensen, 1995; Savory and Griffiths, 1997). The propensity to peck or be pecked may therefore be associated with variations in times spent in preening and other behaviours.

Allelomimetic behaviour is the synchrony or co-ordination of any particular activity (Hurnik *et al*, 1995; Fraser and Broom, 1997b) and is common amongst animals that live in social groups (Fraser and Broom, 1997a). Synchrony of feeding in domestic fowl is well documented (Tolman and Wilson, 1965; Hughes, 1971; Savory, 1975; Keeling and Hurnik, 1993). Preening behaviour in birds is also influenced by allelomimicry. In terns (*Sterna hirundo*), the proportion of birds preening increased with the number of birds present (social facilitation), but was also synchronised, especially among breeding pairs (Palestis and Burger, 1998). Pairs of finches (*Lonchura striata*) synchronised preening behaviour when they were in visual contact with each other (Birke, 1974). As a social species, laying hens are also expected to show allelomimicry.

The presence of a dustbathing substrate in the hen's environment may also be important, since preening often occurs after bathing in some birds (van Iersel and Bol, 1958), (Simmons, 1964). In laying hens, feather lipid concentration increases with dustbathing substrate deprivation, and is then reduced to normal (control) levels once dustbathing substrate is available again (van Liere and Bokma, 1987). Lipid from the preen gland is applied during preening, thus it may follow that preening and preen gland usage are affected by the presence or absence of a suitable dustbathing substrate. After sandbathing substrate deprivation, kangaroo rats (*Dipodomys merriami)* groomed for longer on sand, which was more effective at removing sebaceous lipid, than on woodchips (Borchelt *et al*, 1976). Since most laying hens are currently housed on wire floors, which are unsuitable for functional dustbathing, this could affect preening and dustbathing behaviour.

This chapter addresses a variety of external stimuli that might affect preening behaviour. Experiment 2 uses food frustration techniques to define 'normal' preening as opposed to displacement preening. Once normal preening was established, Experiment 3 addresses the issue of feather pecking (in both pecking and pecked birds)

and its possible effects on preening and Experiment 4 investigates the timing of preening in groups, using data obtained in Experiment 1 (Chapter 3). Experiments 5a and 5b set out to investigate how floor substrate affects preening, due to the association between floor substrate and removal of preen oil from the plumage through dustbathing.

4.2 Experiment 2 - Displacement preening

4.2.1 Introduction

This experiment was intended to distinguish non-functional (displacement) preening, as previously described in conflict/frustration contexts, from functional ('normal') preening. This would be indicated by increased time spent preening overall (Kostal *et al*, 1992; Webster, 1995; Webster, 2000), with a greater number of short preen bouts and more time spent attending to near body sites (here, breast and wings), when frustrated (Duncan, 1970; Duncan and Wood-Gush, 1972a). By comparing preening in a non-frustrating situation (*ad libitum* access at all times) to 2 food frustration situations (food covered in perspex after 4 and 8 h food deprivation), comparisons could be drawn regarding time spent in preening and other activities, sites targeted during preening, number of preen bouts and bout duration.

4.2.2 Materials and methods

Eight ISA Brown hens aged 20 weeks were removed from group cages in a battery house where they had been housed since 18 weeks of age, and relocated to a small room in another building (day –6). After being given a uniquely coloured leg ring, birds were housed in one row of individual cages with a floor area of 1505 cm^2 each. The row of cages was free standing over a concrete floor covered in litter to collect droppings. There were two nipple drinkers and one trough feeder per cage. Cage walls were solid, and flaps protruded out from the cage walls so that birds were visually isolated from each other at all times. Mean light intensity at bird head height was 35 lux, and the mean room temperature was 19° C.

Hens were maintained on a 14-h light cycle and had *ad libitum* supplies of water, and feed when not being tested. For the first week (day –6 to day 0), no experimental work was carried out in order to allow the hens to adjust to their new housing. Hens were weighed on day –6 and on day 0 before testing, and also at the end of all testing on day 6. Top and bottom mandible lengths were measured over two days (days 5 and 6), on a non-test day for each bird. This was to assess variations in beaks as a possible influence on preening behaviour.

Two birds, one at each end of the cage row, were not tested, but were used as 'companion' birds so that all hens tested had birds on each side of them. The 6 remaining birds were tested on each of the three food restriction schedules using two Latin square designs (Table 4.1).

Table 4.1 *Recording schedule of 6 hens subjected to food withdrawal and frustration (4 and 8 h) and control (0 h) treatments.*

	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
Bird 1	0		8		4	
Bird 2	4		0		8	
Bird 3	8		4		0	
Bird 4		0		4		8
Bird 5		4		8		0
Bird 6		8		0		4

On a test day, three neighbouring birds were subjected to one of the three food withdrawal periods (0, 4, 8 h), each bird having a different withdrawal period to either of the other two. After the withdrawal period, each hen in turn was presented with food covered in Perspex for 20 min, during which its behaviour was sampled remotely using a video camera (see Chapter 2 section 2.3.2 for details). For the control (0 h), food was always present (uncovered). All hens were recorded between 17:00 and 18:15 h. After videorecording, each of the three hens tested was allowed *ad libitum* access to food for at least 36 h before being tested on a different food restriction schedule in a similar manner. On the following day, the other three hens were tested and sampled in the same fashion. This procedure was followed three times so that all hens experienced all three treatments.

From the videorecordings (a total of 60 min per bird), all behaviour was continuously recorded according to 8 mutually exclusive categories, in either stand or sit posture. These were preening, comfort activities, feeder directed, drinker directed, spot pecking, head out, walking and 'other' (standing or sitting only, bill wiping). Preening behaviour was further broken down into body sites targeted. All comfort activities seen (head scratching and body shaking) were recorded, whether in close association (< 45 sec, as determined by the bout criterion in section 2.7.1) with preening or not. No wing flapping or pecking at legs or feet were seen.

Bird weights were analysed by paired t-test, comparing weight at day –6 to day 0, day 0 to day 6, and day –6 to day 6. Upper and lower mandibles, and the difference between them, were checked for similarity of variance between all 6 hens and for normal distributions. Behavioural data were calculated in percent time out of 20 min, angular (arcsine root) transformed and analysed using one-way ANOVA, to compare between the three treatments, and also between control (0 h) and combined frustration treatments (4 and 8 h). As well as being analysed individually, time spent at the feeder, drinker, pecking at cage walls, and in preening were also combined to look at time spent in total oral behaviour. The bout criterion interval determined in Chapter 2 section 2.7.1 was applied to preening data and numbers and durations of bouts were compared between treatments, also using one-way ANOVA. All data are presented as means ± standard error, showing original (untransformed) percent values for behaviour.

4.2.3 Results

Body weight (g) ± SE increased over the pre-testing and testing period from 1770 ± 17 on day –6, to 1792 ± 15 on day 0, and 1808 ± 20 on day 6. Mean body weights on day 6 were significantly heavier compared with day –6 ($P = 0.001$, by paired t-test) but not with day 0 ($P = 0.37$). Top and bottom mandible lengths and mandible length differences between birds were also similar, therefore this was ruled out as a factor in preening. Mean mandible lengths (in mm) were 31.9 ± 0.7 for top, 29.8 ± 0.5 for bottom, and 2.2 ± 0.6 for (top – bottom) length difference.

During the 20-min sampling periods, three different hens (two after 0 h and one after 8 h deprivation) did not preen. Most time was spent in feeder directed activity, with the head out of the cage, or in other (Table 4.2). Little time was spent in preening, comfort activities, drinker directed, spot pecking, or in walking. There were no significant differences ($P > 0.05$) or tendencies ($P > 0.10$) between the three treatments (0, 4 or 8 h), or between control (0 h) and combined frustration (4 and 8 h) treatments, for total time (%) spent preening, in comfort activities, feeder directed, drinker directed, in spot pecking, or other. There were also no differences in time spent in total oral behaviour (preening, feeder directed, drinker directed, spot pecking) between the treatments or between control and combined frustration treatments (overall mean 27.7 % ± 4.1). Most behaviours occurred while standing.

Table 4.2 *Overall mean (n = 6) percent of time spent in various activities ± SE, during 20 min frustration after 4 and 8 h food deprivation or control in 6 hens, analysed by one-way ANOVA. Figures presented here are original figures.*

Behaviour	Overall mean (%) ± SE	Significance[1]	
		0 h vs 4 h vs 8 h	0 h vs (4 h + 8 h)
Preening	6.7 ± 1.8	-	-
Comfort activities[2]	0.2 ± 0.1	-	-
Feeder directed	18.8 ± 4.2	-	-
Drinker directed	1.0 ± 0.5	-	-
Spot pecking	1.2 ± 0.3	-	-
Head out	23.9 ± 4.2	*	**
Walking	3.3 ± 0.7	+	*
Other[3]	44.9 ± 4.2	-	-
Standing posture	94.7 ± 2.1	+	*

[1] ** = $P < 0.01$; * = $P \leq 0.05$; + = $P \leq 0.10$; - = not significant ($P > 0.10$).
[2]Comfort activities = head scratching, body shaking. No wing flapping or pecking at legs/feet were observed.
[3]Other = standing only, sitting only, bill wiping.

After birds were subjected to 8 h food deprivation, they showed significantly more head out behaviour (36.1 % ± 7.7) than they did after 0 h deprivation (13.1 % ± 5.9). There were no significant differences between the time spent in head out behaviour after 4 h deprivation (22.6 % ± 5.7) and either the 8 h or 0 h treatments. During frustration

treatments overall (4 and 8 h deprivation), hens showed significantly more head out (29.4 % ± 5.0) and walking behaviour (4.3 % ± 0.9) and expressed significantly more behaviours while standing (98.3 % ± 1.5) than when they had constant access to food (0 h deprivation) (13.1 % ± 5.9 for head out; 1.3 % ± 0.2 for walking; 87.5 % ± 4.3 for behaviours while standing). There were tendencies for birds after 8 h deprivation to show more walking behaviour (4.7 % ± 1.0) than after 0 h (1.3 % ± 0.2), but not compared with 4 h (3.9 % ± 1.4), and to express more behaviours while standing (100.0 % ± 0.0) than either the 0 h (87.5 % ± 4.3) or 4 h (96.5 % ± 2.9) treatments.

There were no significant differences ($P > 0.05$) in times spent preening individual body sites between deprivation treatments, or when comparing control (0 h) to frustration (4 and 8 h) treatments. Of the time spent preening, most was spent in attention to the breast (41.6 % ± 7.5), wings (28.6 % ± 5.9), back (11.7 % ± 2.7) and flanks (11.1 % ± 2.6), with little time spent at the tail (4.8 % ± 2.3) or preen gland (2.2 % ± 1.2). Most preening was performed in standing posture (78.4 % ± 9.1).

When the bout criterion interval (45 sec) was applied, there were no significant differences ($P > 0.05$) in mean number of bouts of preening or mean bout length per hen between any of the three deprivation treatments, or between control (0 h) and frustrated (4 and 8 h) treatments (Table 4.3).

Table 4.3 *Mean number of bouts, and mean bout length (in sec) ± SE, by treatment per hen (n = 6) in a 20 min recording session (by one-way ANOVA).*

	0 h	4 h	8 h
Mean no. of bouts per hen in 20 min	2.2 ± 1.0	3.3 ± 0.7	2.2 ± 0.7
Mean bout length (sec)	52.7 ± 19.8	86.4 ± 32.4	28.3 ± 16.8

4.2.4 Conclusions

There were no indications of displacement preening shown during frustration of feeding after 4 and 8 h deprivation, either in terms of total proportion of time spent in preening, or in proportions of time spent at individual body sites during preening. During frustration treatments, hens were expected to spend more time in preening overall, and a

greater proportion of time preening near (breast, wings) body sites, than when not frustrated (Duncan and Wood-Gush, 1972a). The lack of differences in time spent preening is similar to the findings of Savory and Fisher (1992) who found no significant differences in proportions of time spent preening in restricted-fed layer pullets compared over a 10 week period (age 6 – 16 weeks of age) to those fed *ad libitum* (however, restricted-fed birds were not thwarted from feeding). A greater number of short preen bouts was expected in frustrating situations (Duncan, 1970; Duncan and Wood-Gush, 1972a), however the number of bouts or mean duration per bout, between frustration and non-frustration situations, did not differ here either. Hence, no conclusions can be drawn about how to distinguish functional from non-functional preening. During food frustration treatments (after 4 and 8 h deprivation), hens showed more head out and walking behaviour and expressed more behaviours while standing, indicating a higher level of arousal than when non-frustrated.

In this study, birds were not given a training period, in which they would have learned to expect food after deprivation. However, previous work has shown that this is not necessary to stimulate increased time spent preening in frustrating compared to non-frustrating situations (Duncan and Wood-Gush, 1972b). Likewise, the deprivation periods used in this trial were of sufficient length to cause displacement preening in previous work (Duncan and Wood-Gush, 1972b).

The lack of preening behaviour shown by three of the hens can be partly explained by the short (20 min) sampling period per treatment, as preening behaviour has been shown to be infrequent (Gentle *et al*, 1997; Savory and Mann, 1997; Channing *et al*, 2001) (see also sections 4.5.3 and 4.6.3). If preening occupies less than 10 % of time across the day, then it may be expected that a few birds will not preen during a discrete 20 min recording period.

During the feeding frustration treatments, hens showed increasing activity with increasing deprivation compared to the non-frustrating situation, as is evident by increasing time spent in head out behaviour, walking and performing behaviours while standing. When food deprived and frustrated, Duncan and Wood-Gush (1972b) noticed that hens with a higher motivation to feed (caused by a greater number of training days

combined with longer deprivation periods) expressed a higher mean number of stereotypic movements (characterised by walking and circular head movements), as if the birds were attempting to escape, compared to hens with a lower motivation to feed. Here, head out behaviour might be construed as escape behaviour, as it often involved the hen pushing its head and shoulders out through the bottom of the cage front, and not just holding its head out of the cage. The lack of preening by one hen during 8 h deprivation may thus be partially explained by inhibition due to higher motivation to escape.

No unidentified preening was seen during this study. This was most likely due to the small amounts of preening performed overall, plus enhanced viewing of the hens by lining the concrete floor below the cages with litter. This increased the contrast between the hen's plumage and the wire floor, thus making recording of body sites during preening much easier.

4.3 Experiment 3 - Social effects (feather pecking)

4.3.1 Introduction

The aim of this study was to measure percent times spent in preening and other oral behaviours in birds that were classed as either feather peckers or non-feather peckers, based on previous counts of feather pecks given when in group cages, and in birds that were classed as either feather pecked or non-feather pecked based on plumage damage scores. This was to examine if feather peckers are more active than non-feather peckers, and to determine if birds that are feather pecked spend more time in preening overall, and in using the preen gland, than those that are non-feather pecked.

4.3.2 Materials and methods

A total of 28 previously group-housed (in cages of 4), non-beak trimmed LSL (Lohmann x Single White Leghorn) hybrid hens were housed individually in battery cages at the end of lay (67 weeks of age). Each cage had a floor area of 1600 cm^2 and had solid sides but wire fronts and backs. Other birds not used in this study were also

housed in the same room, so birds were not visually isolated from one another. Hens were maintained on a 12-h light cycle, and mean light intensity at bird head height at the front of the cage was 11 lux.

Half the hens were classed as feather peckers (FP) and half as non-feather peckers (NFP) at 66 weeks of age, based on counts of spontaneous feather pecks given in group cages during observation periods lasting 30 or 60 min (mean number of pecks per hour ± SE: 22.1 ± 8.0 with FP, 0.0 ± 0.0 with NFP, $P < 0.01$, by t-test). Half were classed as feather pecked (P) and half as non-feather pecked (NP), based on plumage damage scores to the neck, wings, back and tail (including the belly) using the method described in Chapter 2 section 2.2.4 at 71 weeks of age. The breast was excluded because feathers here would be damaged at this age due to cage wear. The mean scores (± SE) were: 14.1 ± 0.5 with P, 6.4 ± 0.9 with NP, $P < 0.001$, by t-test). This gave 4 categories of 7 birds each: FP,P; FP,NP; NFP,P; NFP,NP. Bird weights did not differ significantly across the 4 categories ($P = 0.75$, by one-way ANOVA), with mean bird weight (measured once during the week of observation) being 1651 g ± 31.

At 71 weeks of age, each bird was scan sampled for 32 min (in 4, 8-min sessions) with behaviour being recorded instantaneously every 10 sec to measure times spent in 13 mutually exclusive behaviours. These were standing, sitting, walking, feeder directed, drinker directed, preening (plus associated comfort movements), and sham dustbathing. Due to the infrequency of the remaining 6 behaviours during scans (billwiping, spot pecking, non-aggressive pecking, stretching, plus head scratching and body shaking, where they were not associated with preening), these were combined into one category 'other'. When preening occurred, it was recorded continuously, even if it extended beyond the 8-min session, until the end of the bout (45 sec with no preening). On average, this meant an extra 38 sec of sampling time per session. During preening, associated comfort movements (head scratching and body shaking) were also continuously recorded where they fell within 45 sec of preening (wing flapping and pecking at legs/feet were not observed), as defined by the bout criterion interval (see Chapter 2 section 2.7.1). From the results, mean proportions of time (in %) spent in each behaviour were calculated (out of 32 min) and transformed by a derivation of empirical logit ($\ln\{(a + 0.5)/(100.5 - a)\}$). For preening behaviour and associated

comfort movements, mean percent time spent at each site or in comfort behaviour were calculated (out of total time spent preening and in associated comfort movements), and also transformed by empirical logit. Analyses were carried out by two-way ANOVA. Results are presented as original mean proportions (in %) ± standard error.

4.3.3 Results

There were significant differences among the four feather pecking categories for standing, walking, feeder directed and preening, but not for sitting, drinker directed, sham dustbathing or other (Table 4.4). The FP,NP and NFP,P birds showed more time in both preening ($P = 0.006$) and standing ($P = 0.002$) than did FP,P and NFP,NP birds (but not significantly more than NFP,NP birds for preening). Time spent walking was higher in pecked birds (FP,P and NFP,P) than in NFP,NP birds ($P = 0.006$). With feeder directed behaviour, FP,P and NFP,NP birds showed more than NFP,P birds ($P = 0.034$). Overall mean times spent in each activity were standing 33.5 % ± 1.9, sitting 27.4 % ± 3.5, walking 2.8 % ± 0.5, feeder directed 19.7 % ± 2.0, drinker directed 4.6 % ± 0.8, preening 8.9 % ± 1.4, sham dustbathing 1.5 % ± 0.8, and other 1.7 % ± 0.2. Most preening occurred while standing (overall mean 79.0 % ± 6.0) but there were no differences in time spent preening while standing across the four categories. The combination of feeder and drinker directed, and preening, gave a mean total proportion of time spent in oral behaviours of 33.1 % ± 2.3, and there was no significant difference in this measurement between categories (by two-way ANOVA).

From continuous recordings, there were no significant differences ($P > 0.05$) among the 4 categories in percent times spent at each individual body site during preening or in associated comfort movements. Overall means (± SE) were: breast 28.3 % ± 3.4, wings 18.3 % ± 2.4, back 15.0 % ± 2.5, flanks 8.2 % ± 1.2, tail 4.4 ± 0.8, preen gland 0.2 % ± 0.1, unidentified 16.3 ± 2.6, and comfort 9.4 % ± 3.8. Only 7 out of 28 hens showed attention at the preen gland: 0 birds in category FP,P, 2 in FP,NP, 2 in NFP,P, and 3 in NFP,NP.

Table 4.4 *Mean proportions (%) of time spent in different behaviours ± SE from scan sampling, analysed by two-way ANOVA after empirical logit transformation (values shown here are original figures). Within rows, values with the same superscript do not differ significantly (P > 0.05). Birds were classed as feather peckers (FP) or non-feather peckers (NFP) and feather pecked (P) or non-feather pecked (NP).*

					Significance of effects[1]		
Activity	**FP,P**	**FP,NP**	**NFP,P**	**NFP,NP**	**FP vs NFP**	**P vs NP**	**Interaction**
Standing	27.4 ± 2.9^{b}	38.6 ± 2.8^{a}	39.7 ± 2.9^{a}	28.3 ± 4.3^{b}	-	-	**
Sitting	30.3 ± 8.3	19.8 ± 7.3	25.5 ± 5.5	34.0 ± 7.1	-	-	-
Walking	3.9 ± 1.0^{a}	2.2 ± 1.1^{ab}	3.5 ± 0.8^{a}	1.3 ± 0.5^{b}	-	**	-
Feeder directed	23.8 ± 4.6^{a}	19.3 ± 4.5^{ab}	11.2 ± 2.3^{b}	24.8 ± 2.8^{a}	-	-	*
Drinker directed	6.0 ± 1.8	3.6 ± 1.2	5.1 ± 2.0	3.6 ± 1.4	-	-	-
Preening	5.7 ± 3.0^{b}	12.1 ± 2.3^{a}	11.8 ± 2.8^{a}	6.0 ± 2.3^{ab}	-	-	**
Sham dustbathing	2.1 ± 2.1	2.6 ± 2.1	1.0 ± 1.0	0.3 ± 0.3	-	-	-
Other[2]	1.3 ± 0.2	1.8 ± 0.6	2.2 ± 0.7	1.6 ± 0.4	-	-	-

[1] ** = $P < 0.01$; * = $P \leq 0.05$; - = not significant ($P > 0.05$).

[2]Other = billwiping, spot pecking, pecking neighbour, stretching, plus head scratching and body shaking, where they were not associated with preening by the 45 sec criterion interval.

4.3.4 *Conclusions*

Birds studied here were examined for the relationship between feather pecking and plumage damage (as previously shown/acquired when grouped) and preening. It may be that investigating the behaviour of individual hens based on how they behave in groups is inappropriate once they are removed from the original social context. Clearly, feather peckers cannot show pecks at other hens that are preening when they are isolated from them, and likewise hens that have been feather pecked, based on their plumage damage scores, cannot be pecked while isolated from feather peckers. It may be that preening elicits feather pecking not because of some intrinsic attractiveness of the activity itself, but because birds are relatively still during these activities and provide an easy target. This is supported by other work, for instance in laying hens studied from 18 to 35 weeks of age, birds that received gentle feather pecks were usually dustbathing (28 % of all gentle pecks), while birds that received severe feather pecks tended to be feeding (34 % of all severe pecks) (Keeling, 1994a). In another study, layer pullets observed between 24 and 32 days of age were usually resting (49 % of all feather pecking) or standing (37 % of all feather pecking) when they were feather pecked (Wechsler *et al*, 1998). Also, birds may be pecked because they have a disarrayed plumage, which has been shown to attract feather pecking (McAdie and Keeling, 2000), and the disarrayed plumage may, in turn, stimulate preening behaviour in the pecked hen.

Pecked birds did not show more feeding behaviour than non-pecked birds. Although in other studies birds were often feeding when pecked (Keeling, 1995; Savory and Mann, 1997), it does not necessarily follow that pecked birds perform more feeding behaviour than non-pecked birds. Unlike with Keeling (1995) or Savory and Griffiths (1997), here pecked birds did not preen more than non-pecked birds, possibly because prior feather loss reduced the need for preening. Feather pecker hens were expected to be more active (Keeling and Jensen, 1995; Savory and Mann, 1997), but in this study feather pecked birds showed the most walking behaviour. This may be due to learned escape behaviour, from earlier attempts to avoid the attention of feather peckers while in group cages. Propensities to express different types of behaviour would presumably differ

between group and individually housed hens, regardless of feather pecking status, due to the influence of conspecifics on each other's behaviour when housed in groups.

Unidentified preening accounted for a large proportion of preening, due to poor visualisation through the cage front. Proportion of unidentified preening shown here was similar to the level shown in the pilot study (Chapter 2), where front viewing through cages was also used. This could also account for so little time spent at the flanks, as this would be hard to identify if the hen was preening offside to the camera.

4.4 Experiment 4 - Social effects (synchrony)

4.4.1 Introduction

The purpose of this analysis was to determine whether preening among discrete groups of pen-housed laying pullets was synchronised, which would indicate allelomimetic properties affecting the behaviour. The data presented here were derived from Experiment 1 (see Chapter 3).

4.4.2 Materials and methods

Details on the subjects and husbandry are as per Experiment 1. All 12 groups of 8 birds each were scan sampled for 1 – 6 sessions per week over 20 weeks (0 to 19 weeks of age) between 0800 and 1900 h. No observations took place when the birds were 8 weeks of age. During a scan sampling session, the behaviour of every bird in a pen was scored once a minute for 15 min, using the 'on the beep' method (Martin and Bateson, 1993). An equal number of sessions was recorded for each pen per week, giving a total of 972 sessions. Collected data included general behaviour (see Appendix C) such as standing, feeding, scratching at litter etc, but only preening behaviour was of interest here.

Data were collected in a matrix-style format, with the session minute (of which there were 15) running down the y-axis, and the bird identification indicator (by individually coloured leg rings, of which there were 8) running along the x-axis (See Appendix D).

Preening behaviour was noted at specific body sites and in either stand or sit posture. Because these details were irrelevant to this analysis, as were all other behaviours, any form of preening was converted to a 1, and behaviours other than preening were converted to 0. Therefore, the data on preening were kept in the same time and bird order on the matrix of 120 (15 x 8) cells (see Appendix E). This was repeated for all sessions, from which mean proportions (%) of time spent preening per session and per week were calculated.

The analysis of the data was based on a method by Hughes (1971), using a randomisation test. For each session, 1000 permutations of the data were created by computer so that the occurrences of preening by each bird were randomly allocated to different minutes, but so that the total time preening per bird per session remained the same. The minute to minute variation in the number of birds preening at any one time was calculated per 15-min session (observed variance) and compared to the variances of the 1000 permutations (permutated variances). A cumulative variance was also calculated for each week over all sessions and compared to 1000 permutated variances for week. The probability of each permutated variance occurring was compared to the observed variance. Observed variance that was equal to or larger than permutated variances at a probability level of 0.05 indicated synchrony, since this indicated more 'clumping' of preening behaviour than if preening had been randomly spread out over the 120 cells (which would show lower variation). To determine if diurnal rhythms in synchronous preening were occurring, the proportion of time spent preening per hour, and the proportion of synchronous preening per hour, were calculated, and analysed using Pearson's correlation.

4.4.3 Results

Proportion of time spent preening per week varied from 5.2 % (age 0 weeks) to 19.0 % (age 16 weeks) (Table 4.5). There was highly significant ($P < 0.001$) synchrony of preening in all weeks. A total of 25.1 % of 15-min sessions showed significant synchrony at $P < 0.05$ or less: the percent of significant sessions per week are given. Data were obtained in an experiment testing the effects of beak trimming: birds in beak

trimmed pens synchronised preening in 12.2 % of sessions versus in 12.9 % of sessions in non-beak trimmed pens.

Table 4.5 *Mean percent time spent preening and proportion (%) of sessions per week that showed synchrony, with observed vs permutated variance of preening for each week (no observations were held when the birds were age 8 weeks). Observed variance for week is significant at P < 0.001 (by randomisation test).*

Week (bird age)	No. of 15-min observations sessions	Mean time spent preening (%)	Proportions of sessions (%) with significant synchrony[1]	Observed variance for week	Permutated variance for week at P < 0.001 level
0	72	5.2	2.8	33.1	32.4
1	48	6.5	16.7	31.5	27.2
2	48	8.3	10.4	37.8	34.1
3	48	11.7	29.2	59.9	41.5
4	60	11.3	28.3	68.6	48.8
5	12	8.6	33.3	11.0	9.3
6	72	11.8	34.7	100.0	62.9
7	72	11.6	33.3	93.8	57.8
9	60	9.3	25.0	55.9	41.3
10	60	9.8	30.0	71.2	43.5
11	24	13.3	33.3	35.4	24.7
12	48	11.0	27.1	49.5	35.7
13	48	12.7	27.1	64.2	43.1
14	60	12.3	28.3	80.5	52.2
15	60	12.7	31.7	83.4	46.7
16	24	19.0	41.7	58.0	31.6
17	48	15.0	22.9	55.5	44.6
18	48	11.7	25.0	54.3	41.3
19	60	8.3	13.3	50.6	39.4
	972 (total)	**10.8 %**	**25.1 % (means)**		

[1]Sessions were significantly synchronised where the observed variance was greater than the permutated variance at $P \leq 0.05$.

Of the sessions that showed significant synchrony, time spent preening ranged from 3.3 % to 69.2 % (mean 19.1 % ± 0.8). Where synchrony was not evident, preening ranged from 0.0 % to 50.0 % (mean 8.1 % ± 0.3). There was a trend for there to be a greater

proportion of synchronous sessions in sessions that showed a high proportion of preening (Table 4.6).

Table 4.6 *Number of 15-min sessions that showed different proportions of time spent preening, and of those sessions, which proportion (%) showed significant synchrony in preening among birds.*

	Time spent preening (%)						
	0	**< 5**	**5 - 10**	**10 - 20**	**20 – 30**	**30 - 40**	**> 40**
No. of sessions	63	278	219	246	98	43	25
% of synchronous sessions	0.0	4.3	19.6	39.0	48.0	72.1	60.0

The mean times spent in synchronous and total preening per hour were plotted, to look for diurnal rhythms (Figure 4.1).

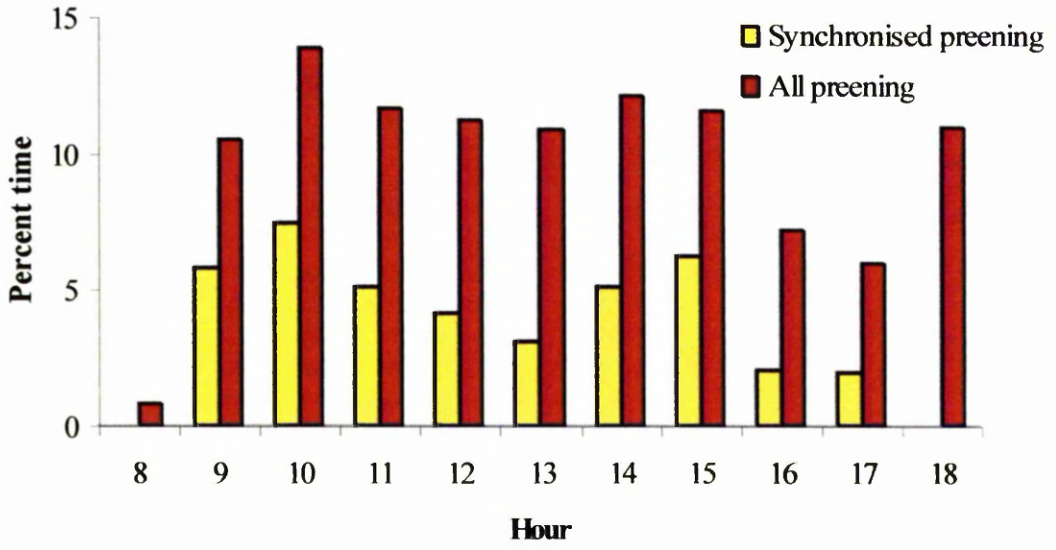


Figure 4.1 *Mean time spent in synchronised and all preening (%) per hour, across the light period.*

There were no sessions with significant synchrony during the hours of 0800 or 1800 h. The time of day of 24 of the sessions was not recorded, due to a failure in recording equipment, thus these sessions are not included in Figure 4.1. The proportion of sessions where preening was significantly synchronised was highest at 1000 h, and lowest at 1600 h. Diurnal rhythms of mean times (%) spent in synchronous and all preening were significantly correlated at P = 0.011 (Pearson's correlation = 0.728).

4.4.4 Conclusions

The results of this experiment indicate that preening in groups of (8) layer pullets can be an allelomimetic behaviour. This is in agreement with Webster and Hurnik (1994) who found that pairs of caged laying hens synchronised preening both within and between cages. Although this was not evident in all 972 (15-min) sessions when analysed individually, it was apparent in all weeks observed, even when birds were very young. Generally, the higher the proportion of time spent preening in 15-min observation sessions, the greater the proportion of synchrony in those sessions. It may be that the small proportions of time spent preening in some sessions make assessing synchrony impossible even where it truly exists, or because preening was randomly allocated over the 15 min in these sessions and was thus not synchronised. Birds showed synchrony in preening from the first week of life, although the percent of sessions with synchrony was lower in this week than in all subsequent weeks, as was the overall mean time spent preening. This may be due to the downy structure of the plumage at that age requiring little attention, or the relatively greater importance of other behaviours in the birds' repertoires at that age, such as feeding or resting.

Determining that preening is allelomimetic does not conclude whether or not it is also enhanced by social facilitation. The mean time spent preening in birds may not only be synchronised but enhanced by the presence of preening conspecifics compared to a bird that preens in isolation (which may in turn be inhibited from preening due to fear; Clayton, 1978, as cited in Nicol, 1995), but only group-housed birds were studied here. Although other experiments presented in this thesis looked at preening in individually housed hens, it is not practical to draw comparisons in preening between grouped and individually housed birds here due to the difference in sampling methods, methods of

housing, possible room effects, and age effects. Though Nicol (1989) observed that the visual presence of pen mates significantly increased preening in individual hens, the simultaneous behaviour of pen mates was not recorded while recording the behaviour of the test bird and thus neither social facilitation nor allelomimicry of preening can be positively confirmed.

Synchronisation of preening behaviour is expected to be an evolved survival mechanism. During preening, birds are exposed to attack by predators since their eyes are closed while they give attention to the plumage with their beaks. This is interrupted by periods of vigilance in which their heads are raised from the plumage and their eyes are open. Keeling and Duncan (1991) noted that when bantams and layer strain birds were in a large (110 m x 80 m) enclosure, the flocks occupied the least area and showed the smallest nearest neighbour distance when preening. They reasoned that birds would position themselves closer to others when vulnerable, such as during preening, than when searching for food, in which they would spread apart. Certainly, the proportion of birds preening has been shown to increase with increased group size, while vigilance generally declines (Newberry *et al*, 2001). Keeling (1994b) found that when birds were given varying space allowances (5630, 3000, 1200 and 600 cm^2 per bird), they were closest together when preening in all but the 600 cm^2 space allowance. By being close together while synchronising preening, an individual's chance of becoming a prey target could expect to be reduced because a) the probability of being targeted goes down as the numbers in a group goes up, and b) while birds in a group are preening the probability that at least one bird will be in a vigilant (head raised, eyes open) position while others preen are high. Other studies also mention anti-predator (Birke, 1974) or maintaining vigilance (Palestis and Burger, 1998) as reasons behind synchronising preening behaviour.

Synchronisation of preening may be influenced by (or influence) diurnal rhythms. Wood-Gush (1959) found preening occurred most just after lights on and just before lights off. In the hours observed in this study, there was a peak in total preening behaviour during the hour of 1000, in which there was also a correlated peak in synchronous preening. Because this study is based on data taken from birds observed from day old to 19 weeks old, the times at which lights went on and off were changing

almost weekly. Times of lights on ranged from 0130 – 0730 h and times of lights off ranged from 1730 – 2330 h, but birds were only studied between 0800 and 1800 h. Thus it is not possible here to determine complete diurnal patterns of preening activity.

4.5 Experiment 5a - Floor substrate (first study)

4.5.1 Introduction

This experiment attempted to address how preening and dustbathing in adult hens were influenced by different floor substrates (wire and litter) in relation to preconditioning on the same or a different substrate. Diurnal rhythms of preening and dustbathing were investigated and the bout criterion (45 sec) was applied to preening data.

4.5.2 Materials and methods

Lohmann Brown hens were housed in a poultry house in either individual battery cages (n = 12) or in two group pens (n = 12 hens per pen) at 18 weeks of age. Caged hens were initially housed in individual cages in which they were not visually isolated (see Chapter 2, section 2.3.1), but at 21 weeks of age they were transferred to individual cages with solid sides and wire mesh backs, with no birds behind them. These individual cages had a (wire) floor area of 1100 cm^2 and were each supplied with two nipple drinkers and one trough feeder. In the group pens, there were 12 hens per pen with a total floor area of 3.82 m^2, housed on litter (wood shavings) with one bell drinker and one food hopper. Group housed hens were wing tagged for identification purposes.

In a different poultry house, there were four adjacent test pens in one room, each with a floor area of 5850 cm^2. These pens were constructed of heavy metal gauge wire with solid wooden partitions between pens. Each pen had one nipple drinker and one trough feeder situated inside the pen. A removable wooden floor could be laid down over the wire floor in any of the four pens and covered with 8 cm depth of litter, which gave test floor substrates of either wire or litter. The light intensity at bird head height was 17 lux and the mean temperature of the room during testing was 18° C.

At 25 weeks of age, top and bottom mandible lengths were measured. At 35 weeks of age, 8 birds each from individual wire cages (W) and from group litter pens (L) were selected for the experiment based on beak similarity, as confirmed by t-tests. Overall mean mandible lengths were (in mm ± SE): top 32.6 ± 0.3, bottom 32.7 ± 0.4, and a mean difference between top and bottom mandibles of –0.1 ± 0.3. Half of the birds from each home (preconditioning) environment were tested on wire (W) and half on litter (L) floors, which gave 4 treatments (by home, test floor) of WW, WL, LW, LL (n = 4 hens per treatment). The location of treatments in the group of test pens was chosen at random.

Each day, one of the 16 birds was weighed, relocated to the test environment and given three days to acclimatise to its new surroundings. In order to maintain a constant number of birds in the room at any one time, up to three companion birds were also housed in the test pens. After three days, each hen was video recorded over two days for 16 h (equivalent to one light cycle, as maintained in the home environment), reweighed and then returned to its home environment. Acclimatisation and testing of all 16 birds was carried out over 20 consecutive days, when the birds were 36 – 38 weeks of age.

From the videotapes, all incidents of preening (including associated comfort behaviours, where they fell within < 60 sec of a preen movement, as in Chapter 2 section 2.3.1) and dustbathing (including sham/vacuum dustbathing on wire floors) were continuously recorded. (The bout criterion of 45 sec was calculated after recording and analysis of this data.) Body sites targeted and postures during preening were also recorded. Proportions of times spent preening and dustbathing were calculated per bird out of 16 h. Of the total time spent preening, proportions of time spent at each site was also calculated. Behavioural data were arcsine transformed, and two-way ANOVAs were carried out to determine the effects of home and test environments, and their interaction. Percent times spent preening and dustbathing were presented for every hour across the 16-h sampling period to assess diurnal rhythms. Results are presented as original (untransformed) mean proportions (in %) ± standard error.

The bout criterion interval of 45 sec (see Chapter 2 section 2.7.1), which was calculated using these data, was applied to determine mean number of bouts and mean bout length per bird with each of the four treatments.

4.5.3 Results

Mean body weights between the 4 treatment groups did not differ significantly either before or after testing. However, over all groups, mean body weight ± SE decreased significantly between pre-test at 2107 g ± 65 and post-test at 2031 g ± 66 (P = 0.000, by paired t-test). Birds from individual wire home cages lost more weight (86 g ± 20) than those from group litter home pens (66 g ± 14) (but not significantly so).

Behaviour recording of the first hen (WL) was cut short by 3 h due to disturbance by an escaped hen. Therefore, proportions of time spent in each behaviour were calculated out of 13 h, not 16 h, for this bird. All 16 hens expressed preening behaviour over the sampling period, although one hen (bird 10, LL treatment) appeared particularly fearful as indicated by its vigilant posture and little time spent preening (0.2 %). Mean proportions of time spent preening and dustbathing did not differ significantly with either home (P = 0.53 and 0.73, respectively) or test (P = 0.74 and 0.14) environments, or their interaction (P = 0.70 and 0.94, all by two-way ANOVA) (Figure 4.2). Overall mean proportions of time spent preening and dustbathing across all treatments were 2.0 % ± 0.3 and 0.4 % ± 0.2, respectively.

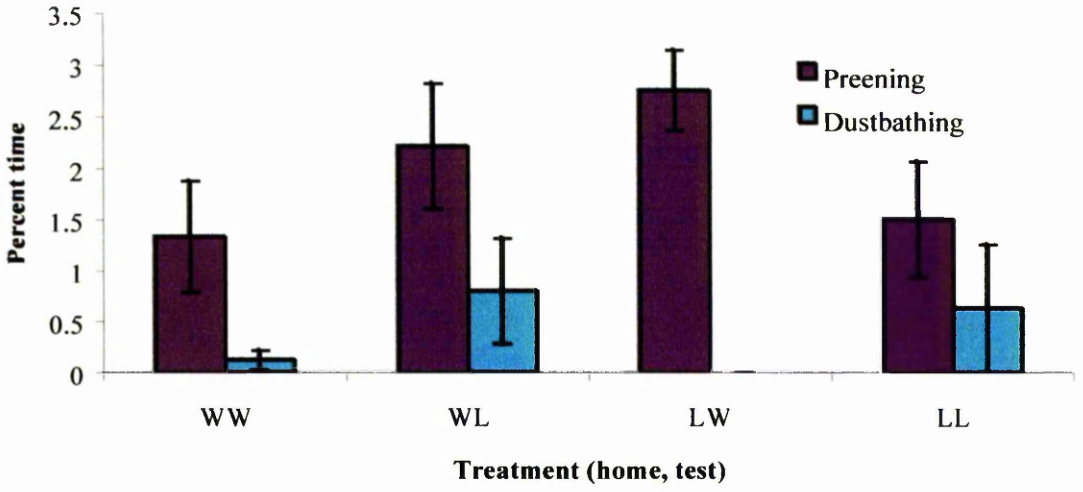


Figure 4.2 *Mean percent of time (out of 16 h) spent preening and dustbathing ± SE, with different treatments (n = 4).*

Hens spent most of their time preening while standing (overall mean 84.2 % ± 4.9) and this was not affected significantly by either home (P = 0.99) or test (P = 0.80) environments, or their interaction (P = 0.60). LW birds showed the most time spent preening (2.8 % ± 0.4) while WW birds showed the least (1.3 % ± 0.6). Of the time spent preening, more than 85 % of the time was spent directed to the breast, wings, back and flanks (Table 4.7). Very little time was spent at the preen gland or tail, or in associated comfort behaviours. All birds except one (bird 10, LL) used the preen gland. Approximately 3 % of preening was at unidentified sites.

Table 4.7 *Overall mean percent time spent preening at each body site or in associated comfort behaviour, out of total time spent in preening and associated comfort behaviour, and the effect of home and test floor substrates (wire or litter), and their interaction, by two-way ANOVA[1]. Figures presented here are original percent ± standard error.*

Preen site	Overall mean % ± SE	Home	Test	Interaction
Breast	25.9 ± 2.8	-	-	-
Wings	21.5 ± 1.8	-	-	-
Back	20.5 ± 1.4	+	-	-
Flanks	17.5 ± 1.3	+	-	-
Tail	2.7 ± 0.5	-	-	-
Preen gland	3.0 ± 0.8	-	-	-
Unidentified	3.0 ± 1.2	+	+	-
Comfort	5.9 ± 1.5	-	-	-

[1] + = $P \leq 0.10$; - = not significant ($P > 0.10$).

There was a tendency ($P \leq 0.10$) for hens from the wire home environments (WW, WL) to show less attention to the back (17.9 % ± 2.4) and flanks (15.2 % ± 2.1) than litter home (LW, LL) hens (23.0 % ± 0.9 and 19.9 % ± 1.1, respectively). Hens on wire with both home (WW, WL) and test (WW, LW) environments spent more time in unidentified preening (4.9 % ± 2.3 and 4.9 ± 2.3, respectively) than litter home (LW, LL; 1.0 % ± 0.4) or test (WL, LL; 1.0 % ± 0.4) hens.

During the recording of dustbathing behaviour, one hen (WW treatment) dustbathed in her food tray. These data (0.4 % of light cycle) were not included in the dustbathing analysis. WL hens spent the most time dustbathing (2.2 % ± 0.6) and LW hens did not dustbathe at all (0.0 % ± 0.0) except for 1 hen sham dustbathing for a total of 9 sec. The numbers of WW, WL, LL, and LL hens that dustbathed were 2, 3, 1, and 1 respectively. All hens that dustbathed were also seen to use the preen gland at some time during preening.

When looking at diurnal rhythms, there were peaks in preening and dustbathing approximately mid-way through the light cycle (Figure 4.3). Preening was fairly consistent at about 1.5 – 3 % of time in the first half of the light cycle, peaked at 1300 h (3.6 %) and 1400 h (3.4 %), and then decreased until lights off. There was virtually no

dustbathing in the first half of the light cycle, and after a peak at 1200 h (3.2 %), dustbathing declined rapidly. There was no dustbathing in the last 3 h of the light cycle.

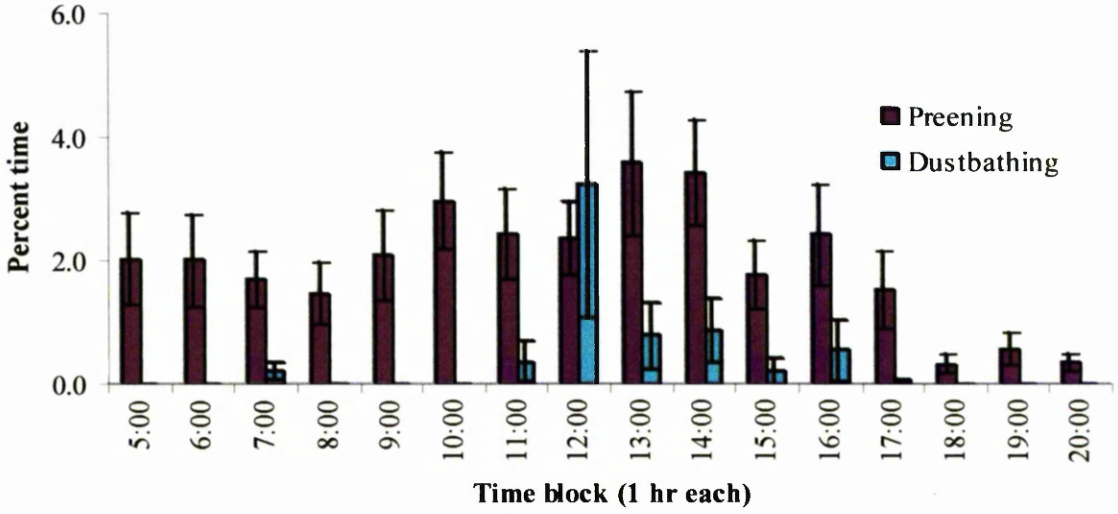


Figure 4.3 *Mean proportion (%) of time spent in preening and dustbathing ± SE by all hens (n = 16) in each hour of a 16 h light cycle.*

The bout criterion interval of 45 sec was applied to all preening and associated comfort behaviour (Table 4.8). There were no significant differences in mean number of bouts per hen or mean bout length between home (P = 0.20 and P = 0.81, respectively) or test (P = 0.46 and P = 0.73) treatments, or their interaction (P = 0.69 and P = 0.49, all by two-way ANOVA).

Table 4.8 *Mean number of bouts, and mean bout length (in sec) ± SE, by treatment per hen (n = 4) in a 16 h recording session (by two-way ANOVA).*

	WW	WL[1]	LW	LL
Mean no. of bouts per hen	24.8 ± 6.3	22.0 ± 3.2	38.5 ± 6.4	29.3 ± 12.4
Mean bout length (sec)	103.2 ± 12.9	129.4 ± 33.3	126.6 ± 22.2	117.7 ± 24.4

[1]One hen sampled for 13 h.

Overall mean number of bouts and mean bout length per hen were 28.6 ± 3.9 and 119.2 sec ± 11.2, respectively. The bout criterion interval of 45 sec, which was assessed using this data, was calculated after analysis of percent time spent preening and in associated comfort movements, in which an arbitrary criterion of 60 sec had been used to define this association. Reviewing the original data sets with the new 45 sec criterion revealed that only 0.75 sec per hen of associated comfort behaviours had been misassigned to being closely associated with preening. This would have altered the results shown in Table 4.7 by reducing mean time spent in associated comfort behaviour by approximately 1 %, and mean time spent preening overall by less than 0.1 %.

4.5.4 Conclusions

Neither home nor test floor substrate, nor their interaction, affected overall percent times spent preening and dustbathing over one light cycle. This may have been affected by the variation seen within each treatment (WW, WL, LW, LL) (Figure 4.2) due to a) moving birds to an unfamiliar environment, and b) changing the floor substrate for some birds. Black and Hughes (1974) found more total time spent preening in group caged birds, but more time in other comfort behaviour (wing flapping, move flaps, and stretching) in birds housed in group litter floor pens (space per bird was greater in group pens). Also, when birds were transferred from cages to pens and *vice versa* in Black and Hughes's (1974) study, the rate of preening did not alter, but wing flapping increased from cages to pens and decreased from pens to cages. Nicol (1987) found that moving hens from small to large cages resulted in rebound effects (increased rate of performance in the large cages compared to that shown in the small cages) on preening and wing flapping. A change in available space may therefore be more important than

presence of litter in affecting preening and comfort behaviour. Here, for all hens, floor space per hen increased with the movement from the home environment to test pens. This may have caused increases in preening and comfort behaviour across all hens that obscured any effect due to floor substrate.

The treatment group that showed the highest percent time preening (LW) did not also show the highest percent time dustbathing (WL). Testing birds in a different environment (floor type; WL, LW) may have complicated the results due to rebound effects in WL hens, and inhibition effects in LW hens, on dustbathing behaviour. Black and Hughes (1974) showed similar results when moving hens from wire cages to litter-floor pens (which increased dustbathing) and *vice versa* (dustbathing ceased). Previously, authors have shown the effects of depriving hens of a suitable dustbathing substrate for long periods (as in WW hens), resulting in sham dustbathing during deprivation (van Liere and Bokma, 1987; van Liere and Wiepkema, 1992; Petherick *et al*, 1995), and that providing deprived birds with a friable substrate (as in WL hens) results in increased time spent dustbathing compared to non-deprived hens (van Liere and Bokma, 1987). Moving hens from a preferred to a non-preferred dustbathing substrate (as in LW hens) has been shown to inhibit dustbathing (van Liere *et al*, 1990). Similar results were evident in this study, though not to a significant extent.

The tendencies to show lower proportions of time spent preening at the back and flanks in wire compared to litter home hens are not easily explained. It may be that in wire floor cages, accessing the flanks is more difficult than in litter floor pens used here, due to available space. Dawkins and Hardie (1989) have shown that, during preening, hens of approximately 2 kg in weight (as in this study) occupy up to 1977 cm^2 of floor area, which is larger than the space allowance used in wire home cages here. To preen the flanks, hens expose the area by holding the wings away from the body. In individual cages, this may have been inhibited if hens were reluctant to touch the wings unnecessarily to the cage surfaces, which may cause feather abrasion (Cate, 1985).

Peaks in both preening and dustbathing occurred mid-way through the light cycle, suggesting a causal link between them. It was expected that those hens tested on litter might perform fewer, longer preening bouts as opposed to those on wire, because of the

association with dustbathing and removal of preen oil (van Liere and Bokma, 1987a), which would then need replenishing. Although this was the case with both mean number of bouts and mean bout length, these differences were not significant. As with preening overall, there was high variation within treatments in number of bouts and bout length, which may be a reflection of true variability between birds with regards to this behaviour, or due to the small sample size.

4.6 Experiment 5b - Floor substrate (second study)

4.6.1 Introduction

In order to simplify comparisons between birds on different substrates with regards to preening and dustbathing, this study housed birds on the same substrate they were to be observed on, and (unlike Experiment 5a) there was no move from a home environment to a test environment. Because pullets are usually floor (litter) reared in groups, a period of 8 weeks was given to allow birds to adjust to living in isolation and, in some cases, to wire floors, prior to any data collection. Also, a larger sample size was used per treatment, in an attempt to reduce the variation in results, which may have prevented significant differences in the first floor substrate study. The test environment set-up was improved compared to Experiment 5a, to enhance the video image of the birds (by giving them a smaller floor area, which thus reduced the camera distance) and to prevent dustbathing in food trays.

4.6.2 Materials and methods

At 18 weeks of age, 24 beak trimmed ISA Brown hens were housed in one room in individual pens with either a wire or a litter floor (n = 12 per floor substrate). The number of wire and litter floor pens was distributed equally along two walls, in a random order. Each pen had solid walls so that hens were visually isolated, with a floor area of 1440 cm^2. There were two nipple drinkers, and a trough feeder situated outside a small head pop hole in each pen. The trough feeders had flaps so that hens were visually isolated when feeding also.

Hens were individually weighed on arrival at 18 weeks and distributed evenly across the two floor treatments by weight. They were also all weighed at age 25 weeks prior to any behaviour sampling taking place, and then again after each hen was videorecorded (age 26 – 29 weeks).

Due to the small size of the pens, those hens on litter quickly saturated the litter with faeces and sometimes water from the nipple lines (due to leaking, overdrinking, or manipulation by hens). Thus, half of the litter in these pens was changed for fresh litter, which was mixed in with old litter, twice a week, and always two days prior to each litter hen being videorecorded.

For 8 weeks, hens were subjected to routine husbandry practices, with the light schedule being stepped up from 12.5 h at 18 weeks of age to 14 h at 21 weeks of age, which was maintained for the rest of the study. At 26 weeks of age, videorecording commenced, with one hen being sampled for one light cycle (1000 – 0000 h) minus the first 0.5 h (1000 – 1030 h) during which daily procedures (feeding, egg collection, moving video equipment, weighing hen) were carried out. Mean light intensity at bird head height was 15 lux during behaviour sampling, and mean room temperature was 20° C.

Body weights and beak measurements were compared between wire and litter floor treatments by t-test. From the videotapes, all incidents of preening and dustbathing were continuously recorded. This included all associated comfort behaviours (body shaking, wing flapping, head scratching, pecking at legs/feet) that fell within the 45 sec bout criterion, and those that did not. Body sites targeted and postures during preening were also recorded. Times spent preening and dustbathing were calculated per bird in percent, out of 13.5 h. Of the time spent preening, percent time spent at each body site was also calculated. All behaviour data were analysed by Mann-Whitney U-test, because they did not follow normal distributions and could not be transformed satisfactorily. Percent times spent preening and dustbathing were presented in 45-min blocks across the sampling period to look for diurnal rhythms. All data are presented as original means ± standard error (rather than medians and interquartiles, in order to make comparisons to other experiments easier), or means with standard error of the difference.

4.6.3 Results

Body weights did not differ significantly between wire and litter floor treatments at any age. However, when comparing bodyweight before (age 25 weeks) and after videorecording (26 – 29 weeks), all hens gained weight significantly (1688 g vs 1728 g, SED = 26, with 46 degrees of freedom, P = 0.02, by paired t-test).

There were no significant differences (by t-test) between floor treatments in top or bottom mandible lengths, or the difference between them. The overall mean mandible lengths were 30.7 mm ± 0.2 for top, 32.5 mm ± 0.3 for bottom, and a mean difference of 1.9 mm ± 0.3 between top and bottom mandibles.

During videorecording, one hen housed on litter was sampled for only 8 h, instead of 13.5 h, due to human error. All proportions for that hen were therefore calculated out of 8 h. There were no significant differences (by Mann-Whitney U-test) in mean proportions of time spent preening (3.4 % ± 0.4 and 3.2 % ± 0.6, P = 0.71) and dustbathing (1.3 % ± 0.5 and 1.3 % ± 0.6, P = 0.73) with wire or litter floors, respectively. There was a tendency ($P \leq 0.10$) for hens housed on litter to spend a higher proportion of their time preening while standing (87.3 % ± 4.2) than hens housed on wire (75.3 % ± 5.1). A total of 12 of the 24 birds sampled here performed dustbathing movements (n = 5 for wire, n = 7 for litter).

Of the time spent preening, most attention (91.9 %) was given to the breast, wings, back and flanks (Table 4.9). Very little time was spent at the tail, at the preen gland, or in associated (within < 45 sec) comfort movements or at unidentified sites. All birds except 2 (1 each on wire and litter) used the preen gland at some time during preening.

Table 4.9 *Overall percent time spent preening at each body site or in associated comfort behaviour, out of total time spent in preening and associated comfort behaviour, on wire and litter floor treatments, and the difference between them (by Mann-Whitney U-test). Figures presented here are original percent ± standard error of the mean.*

Preen site	Overall mean % ± SE	Significance[1]
Breast	23.6 ± 1.7	-
Wings	28.9 ± 2.6	-
Back	22.2 ± 1.4	-
Flanks	17.3 ± 1.2	-
Tail	4.7 ± 0.5	-
Preen gland	1.2 ± 0.2	-
Unidentified	0.6 ± 0.2	+
Comfort	1.6 ± 0.3	-

[1] + = $P \leq 0.10$; - = not significant ($P > 0.10$).

There was a tendency ($P \leq 0.10$) for more time to be spent in unidentified preening on wire (1.0 % ± 0.3) as opposed to litter (0.2 % ± 0.1) floors. Associated comfort behaviour (separated by < 45 sec) formed an integral part of preening bouts, accounting for 46.0 % ± 4.4 of all comfort behaviour seen.

There were peaks in both preening (7.3 %) and dustbathing (15.5 %) at approximately midway through the light period (Figure 4.4). Preening fluctuated between 2.2 % ± 0.8 and 4.9 % ± 1.2 in the first third of the light cycle. There was very little dustbathing in the first half of the light cycle (less than 2 % in any time block). Both preening and dustbathing declined rapidly in the last third of the light period. All birds that dustbathed except one (housed on litter) had been seen to use the preen gland during preening.

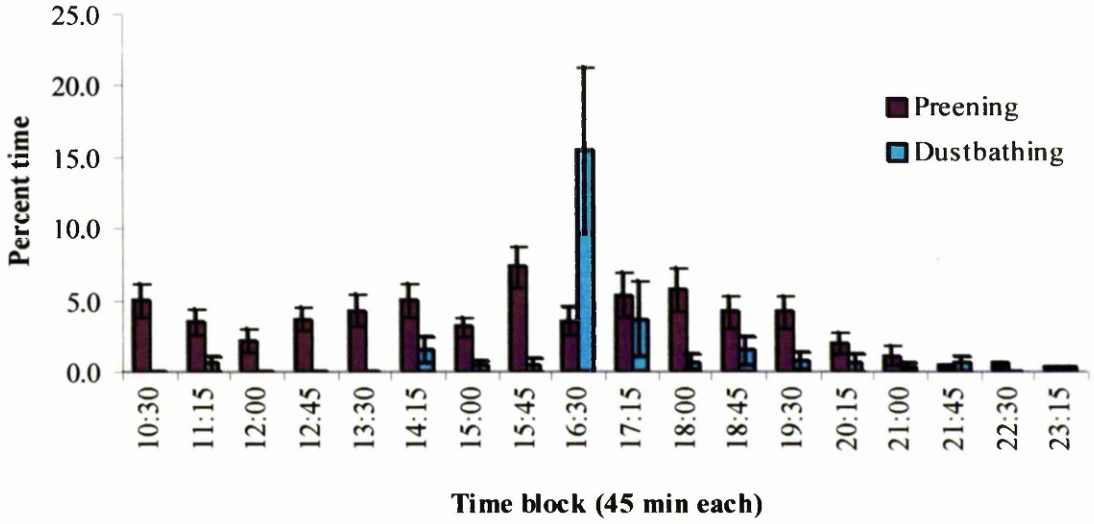


Figure 4.4 *Mean proportion (%) of time spent in preening and dustbathing ± SE by all hens (n = 24) in each 45 min block of a 14 h light cycle.*

4.6.4 Conclusions

Despite having a larger sample size per treatment (n = 12) and a longer settling in period (8 weeks) in this experiment compared to Experiment 5a, there were still no differences in percent time spent preening and dustbathing between floor treatments, and variation among birds was still high. As in Experiment 5a, preening and dustbathing peaked approximately midway through the light cycle. Half of all birds showed dustbathing behaviour in one light cycle.

After at least 8 weeks without access to dustbathing substrate, as opposed to as little as 3 days (LW birds) in Experiment 5a, here wire birds were expected to show less time at the preen gland during preening, due to a presumed build-up of preen gland lipids on the plumage in the absence of dustbathing substrate (van Liere, 1992a). Birds on litter tended to preen more while standing than wire birds, but this did not affect the amount of time spent at individual sites between treatments.

Comfort movements were categorised into those that fell within preen bouts (i.e. within < 45 sec of the preen bout criterion interval) and those that fell without. Just under half

of all comfort behaviours fell within preening, supporting the theory that these behaviours are often associated with preening. Where these behaviours were performed outwith preening, they tended not to be associated with other behaviours, apart from body shaking, which often occurred after dustbathing (pers. obs.). Van Iersel and Bol (1958) considered body and head shaking, scratching, head rubbing and stretching as 'belonging to the preening ceremony' in terns. Studies on poultry vary as to how they categorise these behaviours. Some classify preening and comfort movements separately (Duncan and Wood-Gush, 1972b; Nicol, 1987) or categorise them together as grooming (Williams and Strungis, 1979; Mills and Wood-Gush, 1985). Alternatively, preening and dustbathing along with body and head shaking, head scratching, wing flapping and stretching can all be termed comfort movements (Black and Hughes, 1974).

4.7 Discussion

In Experiment 2, time spent preening the breast in these hens was 13 – 18 % higher, and at the back and flanks generally lower by up to 11 %, than in hens in other experiments presented in this chapter. It may be that frustration levels were so mild as to prevent displacement preening between treatments, but the overall effect of the experiment may be reflecting mild frustration, as is indicated by hens spending a greater proportion of time preening near (breast) as opposed to far (back, flanks) body sites compared to in other experiments here. Although no differences in time spent preening were found among the treatments in Experiment 2, the time spent preening overall (6.7 %) is higher than in the other two experiments (5a and 5b) that used continuous recording techniques. It may be that, although frustration was so low as to be undetectable between treatments, overall preening in Experiment 2 was increased compared to other studies, although comparisons drawn between different experiments must be taken with caution, due to differences in methodology.

Neither Experiment 2 nor Experiment 3 showed increases in times spent in other oral activities (e.g. preening, feeder directed, drinker directed, spot pecking) when birds were prevented from either feeding or feather pecking, respectively. Prevention of one oral behaviour could have resulted in an increase in another or others, as oral behaviours

may sometimes be substitutable (Savory and Maros, 1993). For instance, in broiler breeders, restricted-fed birds showed greater proportions of time in stereotypic pecking at empty feeders or drinkers after the meal was consumed compared to pre-meal levels, which in turn led to increased time spent preening and in litter directed activity if one or both objects being pecked were removed (Kostal *et al*, 1992; Savory and Kostal, 1996). Other animals may also exhibit oral stereotypies when feed restricted, such as sows (Lawrence and Terlouw, 1993). Also, in both Experiments 2 and 3, the overall time spent in total oral activities did not differ between treatment groups within each experiment, which is in agreement with Savory and Fisher (1992), who found that restricted and *ad libitum* fed layer pullets did not differ significantly in total time spent pecking at food and non-food objects.

The proportions of time spent preening and dustbathing were higher in Experiment 5b than in Experiment 5a, which may have been influenced by birds in Experiment 5a being moved from home to test housing. Despite being given three days to adjust to their new surrounding, the move may have caused some hens (particularly those from group pens) to be fearful, remain more vigilant, and perform less behaviour such as preening or dustbathing during which they may feel more vulnerable. Preening behaviour has been shown to increase with group size (Newberry *et al*, 2001), presumably at least partly because birds feel less vulnerable in larger groups. Within both Experiments 5a and 5b, there were no significant differences in times spent preening with different floor substrates. This is in agreement with Nicol *et al* (2001) who found that birds at 186 and 208 days of age housed on either wire or litter floors (regardless of prior exposure to wire or litter) showed no differences in frequency of preening. Bareham (1972) found no differences in time spent preening between group (litter floor) pens or individual (wire floor) cages within two strains of hens. However, Vestergaard *et al* (1990), found that junglefowl chicks spent more time preening in barren environment (wire floors only) pens than in rich (sand and grass covered flooring with perches) environment pens. The overall mean times spent preening in Experiments 5a and 5b are much lower than reported elsewhere (Savory and Hetherington, 1997; Savory and Mann, 1997; Channing *et al*, 2001), probably due to the differences in methods of behavioural recording (here, continuous as opposed to instantaneous

recording methods were used). Continuous methods are more accurate, as they record true durations (Martin and Bateson, 1993).

The lack of difference in times spent at the preen gland between the treatments in both floor substrate experiments (5a and 5b) is consistent with the lack of difference in dustbathing. In the two experiments, all of the birds that showed dustbathing behaviour (on wire or litter) also used the preen gland during the same recording period, except one. It may therefore be that dustbathing behaviour is dependent on use of the preen gland or *vice versa*, but that preen gland use and dustbathing are not necessarily associated closely in time. In both floor substrate experiments, only 3 out of 40 hens studied over one light cycle each did not use the preen gland, but dustbathing was seen in approximately half of all birds (19 out of 40), and of those birds, nearly half were on each test substrate (8 on wire, 11 on litter). Peaks in dustbathing occurred midway through the light cycle. All three of these latter findings agree with Vestergaard (1982), who found that all hens in a flock of 14 housed on straw litter dustbathed on an average 5 out of 10 (i.e. once every 2) days and that a peak in dustbathing occurred midway through a 14-h light cycle, and with Vestergaard *et al* (1990), where no differences were seen in frequency of dustbathing between red junglefowl housed on wire or sand and dirt floors.

Peaks in preening also occurred at the midpoint of the light cycles for both floor substrate experiments, however the peak in preening in Experiment 5a followed the peak in dustbathing by one time block (Figure 4.3), whereas in Experiment 5b, the peak in preening preceded the peak in dustbathing behaviour by one time block (Figure 4.4). This close association of both behaviours in time suggests that preening and dustbathing are closely related in hens, as is supported by the work of van Liere and Bokma (1987), van Liere (1992b), by work on other birds (van Rhijn, 1977), and by studies on grooming and sandbathing in mammals (Borchelt *et al*, 1976). The provision of a suitable dustbathing substrate is important in thermoregulation, as a friable substrate that penetrates between feathers and removes stale preen oil results in a fluffy plumage. A non-greasy plumage is more effective at trapping air and therefore hens lose less heat than when housed on a less suitable substrate. Van Liere (1992b) found that peat and sand were better substrates for removing lipid than woodshavings, resulting in lower

back temperatures and thus less heat loss. Hence, for birds housed on wire floors (as in most commercial egg production systems) there is presumably even greater heat loss due to build-up of lipid on the plumage, particularly when the temperature is below the range of thermoneutrality.

Unidentified preening was higher in birds sampled on wire in both Experiment 5a and Experiment 5b. Viewing dark brown hens overhead against a dark background (as with wire floors over trays covered in droppings) made it more difficult to decipher what sites were targeted during preening compared to viewing hens against (pale) wood shavings.

In terms of the proportion of time spent preening across all experiments in this chapter, attention was directed primarily to the breast, then the wings, back, flanks, tail and preen gland. Time spent at the wings was greater than that at the breast in Experiment 5b and time spent at the preen gland was greater than that at the tail in Experiment 5a. High proportions (approximately 70 % or more) of time spent at the breast, wings, flanks and back are partly due to the plumage area to be covered, compared to the smaller tail and preen gland. But it is probably particularly important to give attention to the breast and wings, as the breast feathers may have a tendency to become disarranged through surface contact (while sitting, feeding, and dustbathing), and the wings would be important from an evolutionary perspective for escape behaviour. In the experiments in this chapter, more preening (about 78 to 84 %) occurred while standing than while sitting, which presumably facilitated easier access to more of the body in this posture.

Comfort behaviour ranged from 1.6 to 9.4 % of total preening and associated comfort activity. The smallest proportion was seen in Experiment 5b, in which hens had the least floor area of all the studies presented here of 1440 cm^2 each, while the greatest proportion was seen in Experiment 3, in which end-of-lay hens had 1600 cm^2 of floor space each. Although the study with the greatest space allowance (Experiment 5a) did not result in the highest proportion of comfort activity, the general trend agrees with Black and Hughes (1974) and Nicol (1987) in which increase in space results in increased comfort behaviour, due to greater space in which to wing flap and body shake. Dawkins and Hardie (1989) found that when hens were given 6724 cm^2 floor space,

they took up the most space during wing flapping (mean 1876 cm^2). Associated comfort behaviour (body shaking, pecking at legs/feet, head scratching and wing flapping) is an integral part of preening bouts, as demonstrated in Experiment 5b, where 46 % of all of these comfort activities fell within preen bouts.

Changes in external influences within (3 out of 5) experiments had little effect on preening behaviour overall or on the time spent in preening individual body sites. Among experiments, total time spent preening varied from less than 2 % (Experiment 5a) to almost 9 % (Experiment 3) of the total time observed, but this is most likely due to the different observation methods employed (continuous vs instantaneous recording). Although differences in strain (Black and Hughes, 1974; Hocking *et al*, 1997), age (Hocking *et al*, 1996; Sherwin and Kelland, 1998), and food form (pellets or mash; Savory and Hetherington, 1997; Savory and Mann, 1997) have been shown to significantly affect time spent preening, other differences, such as in group size (Channing *et al*, 2001), floor area per hen (Nicol, 1987), floor substrate (Nicol *et al*, 2001), or constraints on time budgets (Swennen *et al*, 1989) have been shown to have no such effect. It is possible that changes in a combination of environmental factors could have a greater effect on preening than change in a single factor. Alternatively, it may be that preening is so important that external factors that have an effect may only serve to increase the behaviour, not reduce it. Here, the only consistent effect on preening was behavioural synchrony amongst birds housed in small groups (Experiment 4). The lack of significant results in times spent preening, and at times spent in preening each body site, may have been at least partly due to the small proportion of time that preening occupies and high variation among birds, which made the standard error of the mean within each treatment group high.

Chapter 5

Preen Gland Function

5.1 Introduction

Integral to the study of preening in laying hens is the structure and function of the preen (or uropygial) gland, the organ responsible for producing lipid (also known as oil, or wax) that is distributed onto the plumage during preening.

General avian biology texts give an overview of preen gland anatomy and its microscopic structures in the chicken (Lucas and Stettenheim, 1972a; Jacob and Ziswiler, 1982), however these do not show possible effects of bird age. Previous authors have demonstrated hormonal effects on relative preen gland weight. Castrated and non-castrated cockerels injected with testosterone propionate or diethylstilbestrol showed changes in preen gland weights (both relative and absolute) and histological appearance compared to controls (Kar, 1947). Female pigeons injected with estriol over 7 days showed significantly less extractable preen oil per gland than controls, and there was significant secretory cell destruction in the preen glands of the treated group (Manna *et al*, 1983). Consequently, it is reasonable to expect a change in relative preen gland weight due to hormonal changes with bird age in layer pullets. Information on the effects of housing, or on any relationship between preen gland morphology and feather pecking status, are lacking. Preen gland morphology and histology in domestic hens may be related to bird development and perhaps to environmental factors.

Likewise, feather lipid concentration (i.e. mg lipid per g feathers), which is affected by the presence or absence of dustbathing substrate (van Liere and Bokma, 1987), has not previously been compared between bird ages. Feather lipid concentration may be directly influenced by age, either due to changes in preening behaviour, preen gland function, or both.

Preen oil composition has been shown to be affected by bird age (and thus possibly by hormonal state) in chickens, as demonstrated by changes in proportion of alcohol

fractions (diol esters) of preen gland oil (although bird sex was not specified; Kolattukudy and Sawaya, 1974), and possibly even by diet (Apandi and Edwards, 1964), as indicated by differing proportions of methylated fatty acids (although most fatty acids in the preen gland are synthesised *de novo*) (Stevens, 1996). Because of an apparent relationship between feather pecking and preening (Keeling, 1995), it may be that differing proportions of fatty acids in preen oil amongst birds could affect their likelihood to be pecked. The preen gland has commonly been thought to be the only cutaneous gland in birds (Rawles, 1960; Thompson, 1964). However, some authors suggest that the skin also secretes lipid (Bolliger and Varga, 1960; Bolliger and Varga, 1961; Ishida *et al*, 1973). Histological cross-sections of the skin, scales of the feet, comb, and wattles, and parts of follicles and calami of feathers from chickens all had lipoid secretion granules (Lucas and Stettenheim, 1972b). Ishida *et al* (1973) found that feather lipids were of two types, granules which were arranged along the rachis, barbs and barbules, and irregularly shaped masses which were found on the pennae, plumuli and barbs. They concluded that the granular type, which showed free fatty acids when examined histochemically, originated from the skin, and the mass type from the preen gland, since neither the mass-type lipid or preen gland oil contained free fatty acids (Ishida *et al*, 1973). Comparing the composition of lipid extracted directly from the preen gland to that from feathers would contribute to (or contradict) these findings.

Four principle areas were investigated, in relation to bird age, floor substrate, feather pecking and/or feather pecked status, or lipid source (preen gland vs feathers). These were gross morphology (gland weight and dimensions) and histology (both in section 5.2), quantity of lipid found on feathers (section 5.3), and the fatty acid components of preen oil and oil derived from feathers (section 5.4).

5.2 Experiments 5b, 6a, 6b, 6c - Gross morphology and histology

5.2.1 Introduction

These investigations were designed to look at changes in preen gland weight, both absolute and relative to body weight, and dimensions in relation to bird age, floor

substrate, and feather pecking/pecked status. The histological study of one group of birds revealed how the various zones of the gland altered in area with bird age. Both absolute and relative preen gland weights were expected to increase with bird age. The absence of dustbathing substrate (wire floors) was expected to affect preen gland size due to the relationship between preen oil on the plumage and functional dustbathing (i.e. in the presence of litter). Feather pecked hens might also show differences in preen gland size compared to non-pecked hens, due to the possible association between preening and propensity to be pecked.

5.2.2 Materials and Methods

Preen glands from 4 groups of ISA Brown females were used for the data presented here. Three sets of preen glands were collected for gross morphological study only, to examine the relationship between floor substrates or pecked status (based on feather scores) and preen gland weight and size (dimensions). Of these, one set came from hens used in Experiment 5b (n = 12 per floor substrate), and the details on these birds are given in Chapter 4 section 4.6. To compare hens that had been housed on wire or litter floors for a longer period than those in Experiment 5b, 10 ISA Brown hens each were removed at end of lay from a perchery system with litter floors at age 69 weeks, and a battery system (with wire floors) at age 70 weeks (Experiments 6a). Both sets of hens had been in their respective types of housing for 1 year, and lived in groups of 100 in the perchery and groups of 4 in cages. Light intensities and light cycles were 8 – 10 lux at floor level and 16 h (respectively) in the perchery, and 2 – 10 lux at the front of cages and 14 h in battery cages. All hens had *ad libitum* access to water and a commercial layer mash. To compare preen glands between hens with different feather pecked status, 20 hens from the same battery system flock as in Experiment 6a were selected at end of lay as feather pecked or non-feather pecked, on the basis of pecking damage scores at age 71 weeks. This gave 10 hens in each category (mean feather score ± SE: pecked 21.6 ± 0.5, non-feather pecked 7.3 ± 0.7) (Experiment 6b).

The fourth set of hens was used for both gross morphology and histology, to examine effects of age on preen gland development (Experiment 6c). Here, birds were group-reared in one litter floor pen from day old to 30 weeks. The light cycle followed the

breeding company's guidelines (reduced from 22 h to 16 h over the first week, then reduced by 1 h weekly to 10h, which was maintained from 7 to 14 weeks of age, then gradually increased to 16 h from 14 to 25 weeks of age, at which it was maintained for the rest of the study) (Hubbard ISA, 1998). This resulted in egg production beginning at 17 weeks of age. Mean light intensity at bird head height was 22 lux and temperature was 22° C. The diet was changed from (standard) starter mash to grower mash at 7 weeks of age, and from grower to layer mash at 16 weeks of age. Every 5 weeks, from 5 to 30 weeks of age, 8 hens were randomly selected for preen gland removal. All glands were used for gross morphology data, and 4 – 5 glands were intended for histological analysis. At age 15 weeks, 4 glands were used for preen oil composition analysis.

With all four sets of birds, birds were weighed, killed by lethal injection, and preen glands removed using the method described in Chapter 2 section 2.4.1. Means on gross morphology data were calculated per treatment group ± standard error, checked for normal distributions, and analysed by t-test or one-way ANOVA. The methods used to histologically prepare and examine glands are described in sections 2.4.2 and 2.4.3. Differences in preen gland zone areas between bird age were analysed using REML, due to unequal sample sizes, after confirming that the data were normally distributed. The model compared sections (2 – 5 per gland, n = 107 total) per age, using bird identification as blocking structure, and took into account within-bird variation. Mean values of each gland zone area and total area ± SE were calculated per age (2 – 5 glands per age). Preen glands from birds in Experiment 6b were also assessed as being firm or pliable to the touch when handled, using regression analysis.

5.2.3 Results

With birds housed on different substrates (Experiments 5b and 6a) or classed as feather pecked (P) or non-feather pecked (NP) (Experiment 6b), there were no significant differences in mean body weight between groups within each experiment (Table 5.1). Mean preen gland weight was significantly higher in birds housed on wire in Experiment 6a than those housed on litter. Preen gland weight as a percentage of body

weight was not affected by floor substrate or by feather pecked status. Preen glands between birds of different pecked status (Experiment 6b) did not differ in how firm or pliable they were to touch (by regression, $P = 0.62$, $n = 3$ and $n = 4$ firm glands for pecked and not pecked birds, respectively). In the age comparison experiment (6c), body weight increased with age, as did mean preen gland weight. As a percentage of body weight, the proportion was greatest when the birds were youngest (age 5 weeks). Preen glands from older birds (30 weeks) were firmer to the touch than those from younger birds (pers. obs.). With preen gland dimensions, the heavier preen glands from the heavier (wire) birds in Experiment 6a were also significantly larger in width and length compared to those birds on litter. All preen gland dimensions increased with preen gland weight and bird age (Experiment 6c).

Table 5.1 *Mean body weights, preen gland weights, preen gland as a percentage of body weight and preen gland dimensions with different treatments (floor substrate or age) ± standard error. Analysis by t-test or one-way ANOVA. Different superscripts within a column, within a group (separated by a line) denotes significantly different. For mean body weights and preen gland dimensions in Experiment 6c, significance is at $P < 0.001$; for all others, significance is at $P < 0.05$.*

Expt. No. and treatment	Bird age (wks)	Floor substrate	n	Mean body weight (g) ± SE	Mean preen gland weight (g) ± SE	Preen gland as a percentage of body weight ± SE	Preen gland dimensions (in mm ± SE)		
							Width	Depth	Length
5b	30	Litter	12	1728 ± 32	1.90 ± 0.16	0.11 ± 0.01	22.5 ± 0.5	10.0 ± 0.1	19.3 ± 0.4
(floor)	30	Wire	12	1729 ± 24	1.87 ± 0.10	0.11 ± 0.01	22.8 ± 0.7	10.0 ± 0.3	19.7 ± 0.5
6a	69	Litter	10	1887 ± 57	2.25 ± 0.22^{b}	0.12 ± 0.01	23.5 ± 0.6^{b}	10.9 ± 0.5	20.8 ± 0.6^{b}
(floor)	70	Wire	10	1962 ± 27	2.86 ± 0.15^{a}	0.14 ± 0.01	25.4 ± 0.5^{a}	11.6 ± 0.2	22.6 ± 0.4^{a}
6b	71	Wire (P)	10	1848 ± 40	1.67 ± 0.13	0.09 ± 0.01	21.4 ± 0.6	9.8 ± 0.3	19.2 ± 0.6
(P vs NP)	71	Wire (NP)	10	1970 ± 59	1.62 ± 0.14	0.08 ± 0.01	21.0 ± 0.6	9.2 ± 0.4	20.3 ± 0.4
	5	Litter	8	414 ± 16^{d}	0.58 ± 0.04^{e}	0.14 ± 0.01^{a}	15.1 ± 0.3^{d}	6.3 ± 0.3^{d}	13.4 ± 0.3^{d}
6c	10	Litter	8	1063 ± 25^{c}	0.90 ± 0.04^{de}	0.09 ± 0.00^{bc}	17.7 ± 0.3^{c}	7.1 ± 0.2^{cd}	16.1 ± 0.2^{c}
(age)	15	Litter	8	1353 ± 33^{b}	0.99 ± 0.05^{cd}	0.07 ± 0.00^{c}	18.0 ± 0.3^{c}	7.8 ± 0.3^{bc}	16.7 ± 0.5^{c}
	20	Litter	8	1727 ± 38^{a}	1.27 ± 0.05^{bc}	0.07 ± 0.00^{c}	20.0 ± 0.3^{b}	8.5 ± 0.3^{ab}	17.4 ± 0.2^{bc}
	25	Litter	8	1749 ± 38^{a}	1.47 ± 0.10^{ab}	0.08 ± 0.01^{bc}	21.0 ± 0.5^{ab}	9.0 ± 0.3^{ab}	18.2 ± 0.3^{ab}
	30	Litter	8	1774 ± 22^{a}	1.74 ± 0.14^{a}	0.10 ± 0.01^{b}	22.8 ± 0.8^{a}	9.1 ± 0.4^{a}	19.4 ± 0.3^{a}

When attempting to section glands for histological examination, some did not cut well, resulting in incomplete (and thus unusable) sections, and from age 15 weeks, 4 of the 8 glands per week were used for preen oil composition analysis (see section 5.4), so no spare glands were available for sectioning after this age. Therefore, 2 – 5 glands per age with 2 – 5 sections per gland were obtained for analysis, with a total of 23 glands and 107 sections. An example of a preen gland section is shown in Plate 5.1.

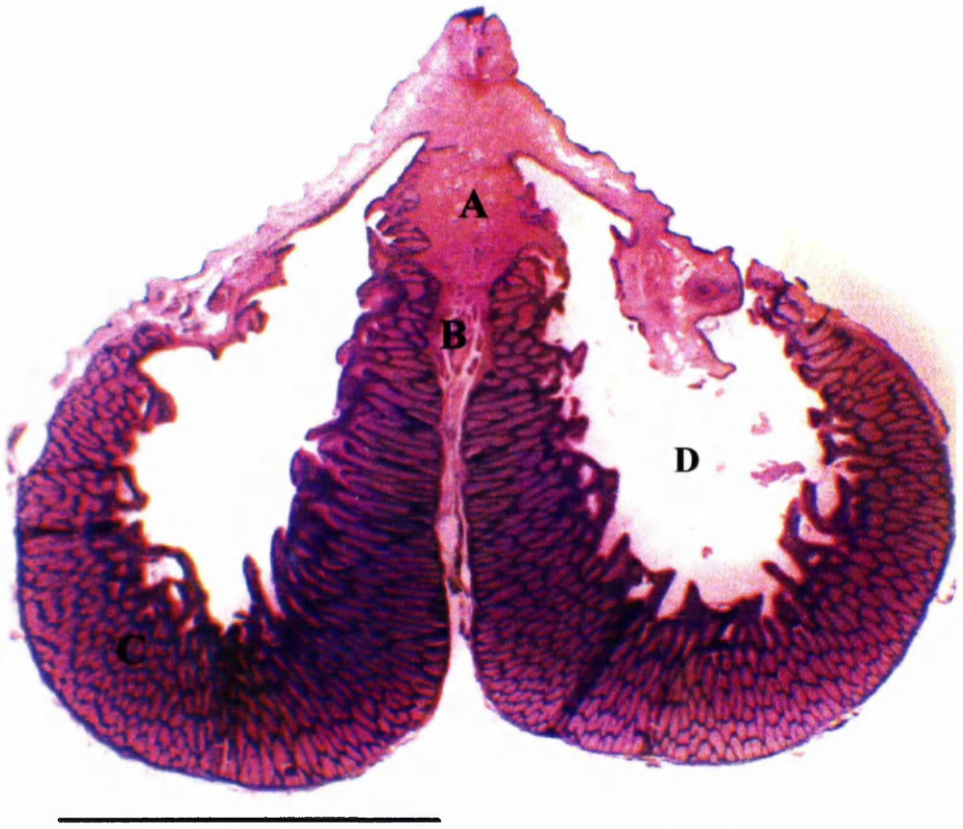


Plate 5.1 *Photograph of a preen gland section from a five-week-old ISA Brown pullet, viewed at x15.75 magnification. Scale bar indicates 5mm. Identified sections were A, papilla (including isthmus); B, interlobular septum; C, capsules; D, cavities.*

The number of glands sectioned per bird age were: 5 at 5 weeks, 5 at 10 weeks, 4 at 15 weeks, 4 at 20 weeks, 2 at 25 weeks, and 3 at 30 weeks. When stained and viewed, the capsules, which produce preen oil, occupied the greatest proportion of the total preen gland area (mean proportion of total over all ages ± SE, 67.8 % ± 1.9). The papilla (13.4 % ± 0.7 of total), which protrudes from the skin and from which the oil is collected, was distinguished from the interlobular septum (6.0 % ± 0.7 of total) as follows: all matter stained grey between the capsule areas of the two lobes was defined as interlobular septum, all that above the capsules (including the isthmus) was measured as papilla area. The two ducts in the papilla, which drain the cavities (12.8 % ± 1.9 of total), were often clearly visible, and one gland had four ducts.

The capsules and the interlobular septum both increased consistently in area with bird age (Table 5.2). The cavities remained statistically similar until week 30, when the area approximately doubled compared to weeks 5 – 20. Papilla area increased between age 5 and 10 weeks, and then remained fairly constant thereafter. Total preen gland area also increased consistently with bird age.

Table 5.2 *Mean preen gland zone areas ± SE, in mm^2, measured using histological methods, and their significance (P value) with sections per bird age (Experiment 6c). Analysis by REML, with WALD statistic and F value given.*

	Preen gland site area (in mm^2 ± SE)				
Bird age	**Papilla**	**Interlobular septum**	**Capsules**	**Cavities**	**Total**
5 (n = 22)	9.8 ± 0.6^{b}	2.5 ± 0.6^{c}	55.7 ± 5.5^{c}	15.5 ± 3.2^{b}	83.5 ± 4.0^{e}
10 (n = 23)	17.3 ± 1.3^{a}	7.1 ± 2.1^{bc}	78.0 ± 7.0^{bc}	12.9 ± 5.2^{b}	115.3 ± 5.5^{cd}
15 (n = 20)	19.5 ± 1.8^{a}	5.8 ± 0.8^{bc}	74.2 ± 7.4^{bc}	11.4 ± 3.8^{b}	111.0 ± 3.6^{d}
20 (n = 19)	16.9 ± 1.5^{a}	9.4 ± 2.2^{b}	105.6 ± 8.4^{a}	7.0 ± 2.8^{b}	138.9 ± 8.5^{b}
25 (n = 10)	16.4 ± 1.9^{a}	11.5 ± 2.9^{ab}	90.7 ± 22.8^{ab}	18.1 ± 13.3^{ab}	136.6 ± 8.5^{bc}
30 (n = 13)	17.0 ± 1.1^{a}	16.2 ± 3.9^{a}	113.8 ± 11.3^{a}	31.9 ± 2.7^{a}	178.8 ± 10.2^{a}
WALD	34.5	25.6	31.7	14.4	119.2
F[1]	6.9	5.1	6.3	2.9	23.8
P	< 0.01	< 0.01	< 0.01	< 0.05	< 0.01

[1]Week d.f. = 5; Bird d.f. = 22; Stratum bird variance d.f. (bird d.f. – week d.f.) = 17.

5.2.4 *Conclusions*

The range of relative preen gland weights (as a percentage of body weight) shown here, with various treatments and ages, are similar to those reported by Jacob and Ziswiler (1982) for various gallinaceous species other than *Gallus domesticus*, which was not included in their study. Values here for layers are lower than those reported for relative preen gland weight in male and female broilers at 4 – 7 weeks inclusive (mean 0.2 %) (Brake *et al*, 1993). There was little variation in relative preen gland size within groups. Increases in mean preen gland weight and size (with all dimensions) were closely linked to bird age and therefore body weight, with relative preen gland weight remaining fairly stable after 5 weeks. This is similar to a study on broiler males and females to 7 weeks of age by Brake *et al* (1993), in which relative preen gland weight remained constant over 4 – 7 weeks in females, and over 4 – 6 weeks in males.

Floor substrate had little effect on absolute or relative preen gland weight when the duration on the respective substrate was short (12 weeks, Experiment 5b) but a longer duration of a year gave a significantly larger mean preen gland weight in wire birds compared to litter birds (Experiment 6a). This is most likely due to hens kept in wire cages having a larger mean body weight (but not significantly so), as relative preen gland weight was not different between the two groups. Whether birds were feather pecked or not was seemingly unrelated to absolute or relative preen gland weight, dimensions, or pliability. However, preen gland characteristics do not necessarily influence preen gland use (or *vice versa*). Within a species, hormonal factors associated with season and sex are perhaps more likely to influence relative preen gland weight (Jacob and Ziswiler, 1982) than the factors tested here.

It appeared that the preen gland was becoming firmer with bird age in which birds were housed on litter (Experiment 6c), since some glands in birds age 30 weeks were more rigid than those from younger birds. Glands were also firm in some 71-week-old hens housed in wire cages for a year (Experiment 6b), regardless of feather pecked status.

The various structures which make up the preen gland increased in size in conjunction with bird age, and therefore with preen gland weight and size. The overall mean capsule area from 5 – 30 weeks of age as a proportion of the whole gland (67.8 %) is

similar to those reported by Jacob and Ziswiler (1982) for other gallinaceous birds (mean of 7 species: 57 %). As the gland's function is to produce oil for distribution over the plumage, it is expected that the oil-making capsules should occupy the greatest area of the gland, as they did here. Normally, domestic fowls have two preen gland ducts in the papilla (Lucas and Stettenheim, 1972a; King and McLelland, 1975). Here, however, a gland with 4 ducts was observed. This is not surprising, as the number of ducts within a species has been seen to vary (Jacob and Ziswiler, 1982).

Difficulty experienced in successfully cutting some preen glands may be partly explained by problems encountered in the preparation technique. Glands were processed whole, making infiltration of reagents difficult. During tissue processing, glands finished in a wax bath, after being in two clearing agent baths. If traces of clearing agent were left in the glands prior to wax embedding, this would cause the tissues to shrink, making blocks difficult to cut, therefore several changes of wax are recommended (Gray, 1972). The wax used to embed the glands was overheated to 65° C (melting point was 57° C), which can harden samples, making them difficult to section (Gray, 1972). Further difficulties can be attributed to gland size (particularly those from birds age 25 or 30 weeks of age), as these glands were most likely to be poorly infiltrated by, and embedded in, wax. Although the largest mould available was used, larger glands often touched the edge of the mould, preventing wax from surrounding the gland properly. Sections obtained by larger glands were therefore more likely to disintegrate on the microtome. It may be that preen gland tissue in older birds was too hard to cut without crumbling.

5.3 Experiments 1, 5a, 5b, 6c - Quantifying feather lipid

5.3.1 Introduction

The quantity of oil on plumage may be indicative of either floor substrate quality and/or variation in time spent using the preen gland (although some lipid on the plumage may be derived from sebaceous secretions from the skin, see section 5.1). Floor substrate in which birds can dustbathe (such as woodshavings) and therefore remove feather lipid

effectively would presumably result in relatively low plumage lipid levels (van Liere and Bokma, 1987), whereas an unsuitable dustbathing substrate (such as wire floors) would result in relatively high feather lipid levels. Effects of age and beak treatment (trimmed vs not trimmed) were also investigated because they may influence the effectiveness of time spent at the preen gland, and hence feather lipid levels.

5.3.2 Materials and Methods

Feathers were collected from four different groups of birds (Experiments 1, 5a, 5b, and 6c), whose husbandry has already been described in previous sections. Feather collection and lipid extraction were carried out using the method described in Chapter 2 section 2.5 in order to give feather lipid concentration (mg per g feathers). Petroleum ether (a non-polar solvent) was used (on freshly-cut feathers) as the extractant in all but Experiment 5b, in which 2:1 chloroform/methanol (a more polar solvent) was used (on feathers frozen for up to 5 weeks at -20° C), in order to elute a greater quantity and variety of lipids. Petroleum ether was recommended as an extractant in a Soxhlet apparatus (Pearson, 1970; Pearson, 1991; James, 1995), and had been previously shown to be an effective extractant for feather lipids (van Liere, 1992a), however the amount of material extracted has been shown to increase with increased extractant polarity (Harrison, 1939, as cited in Pearson, 1991). This was confirmed by doing a pairwise comparison between the two types of extractants on feathers from 3 litter-housed hens from Experiment 5b: the chloroform extractant removed significantly more substance (27.8 mg ± 0.4) per g feathers than did ether (10.4 mg ± 0.6) (by paired t-test, P = 0.000). Oil on feathers from 8 hens each housed on wire and litter in Experiment 5b was extracted using 2:1 chloroform/methanol at age 26 – 29 weeks. The concentration between the two groups was not significantly different (26.8 mg/g ± 1.2 for litter, 28.5 mg/g ± 1.1 for wire). This is most likely because 2:1 chloroform/methanol would have extracted other substances besides lipids (such as proteins, salts, sugars, and amino acids), which would mask the true results. The solution obtained after extraction should have been filtered and washed before drying, in order to remove these unwanted substances, thus these results are not discussed in further detail due to errors in the method. Alternatively, because the litter was being changed twice a week due to build

up of droppings and wet litter (see section 4.6.2), the litter was never left long enough to break down into smaller particles, as would happen through bacterial action in litter pens left for several weeks. Fresh woodshaving size may have prevented their penetration into the plumage, and their total surface area, thus the ability to absorb oil may have been diminshed. Therefore, the lack of difference in feather lipid concentration between the two floor treatments in Experiment 5b may have been partly exacerbated by the husbandry methods.

With feathers from the remaining 3 groups of birds, comparisons were made between different ages (Experiments 1 and 6c) different beak treatments (Experiment 1), and different floor substrates (wire vs litter, after at least 47 weeks (Experiment 5a) on the respective substrate). Data were analysd by one-way ANOVA or t-test, with mean concentrations ± standard error, or means with SED, shown.

5.3.3 Results

Bird age had very little effect on feather lipid concentration (Table 5.3). In birds studied from 5 to 20 weeks of age, the concentration was highest at 5 weeks, and in birds studied from 15 to 30 weeks, it was highest at 30 weeks. Concentrations at 15 and 20 weeks were similar in both age effect experiments.

Feather lipid concentration was affected by floor substrate, as judged by the results from Experiment 5a, where it was significantly higher in birds housed on wire compared to those on litter (both sets of hens had been on their respective substrates for almost 1 year). In Experiment 1, pens of birds were either beak trimmed or non-beak trimmed (n = 6 for each treatment). This had no effect on mean feather lipid concentration (7.9 mg/g for BT, 7.7 mg/g for NBT, 0.6 SED, with 10 degrees of freedom), pooled across all 4 sampling periods (5, 10, 15, and 20 weeks of age) (P = 0.69, by t-test).

Table 5.3 *Mean feather lipid concentration (mg lipid per g feathers) according to treatment (age or floor substrate) ± standard error. Analysis by one-way ANOVA or t-test. Different superscripts within a column, within a group (separated by a line) denotes significantly different at P < 0.001.*

Experiment no.	Bird age (weeks)	n	Floor substrate	Mean feather lipid level (mg) per g feathers ± SE
1	5	12	Litter	10.0 ± 0.5^{a}
	10	12	Litter	6.3 ± 0.4^{b}
	15	12	Litter	6.4 ± 0.5^{b}
	20	12	Litter	8.2 ± 0.2^{b}
6c	15	6	Litter	5.8 ± 0.4^{b}
	20	6	Litter	7.6 ± 0.2^{b}
	25	6	Litter	10.9 ± 1.3^{b}
	30	6	Litter	17.3 ± 2.4^{a}
5a	65 – 67	6	Litter	9.7 ± 0.8^{b}
	65 – 67	6	Wire	28.5 ± 2.4^{a}

5.3.4 Conclusions

Investigation of the effects of age on feather lipid concentration showed that levels were significantly higher at 5 weeks of age than at 10, 15, or 20 weeks (Experiment 1). However, this may have been affected by the presence of pulp at that age in the growing feather calamus (King and McLelland, 1984), which may have also been extracted. Feather lipid levels were highest at 30 weeks in Experiment 6c. This may indicate greater preen gland usage and hence lipid distribution over the plumage, and/or greater sebaceous secretions from the skin, at this age compared to other ages.

When hens were kept on either wire or litter floors for almost a year, birds housed on wire gave significantly higher concentrations of feather lipid than those housed on litter. This is in agreement with van Liere and Bokma (1987), who found that when hens were deprived of dustbathing substrate for 33 days, mean feather lipid concentrations significantly increased compared to pre-deprivation levels or levels in control hens. Mean feather lipid concentration for birds housed on litter in Experiment 5a (9.7 mg/g) are similar to van Liere and Bokma's (1987) levels in pre-deprivation and control hens (10.3 and 9.6 mg/g, respectively), however concentration after only 33 days deprivation

was only 14.5 mg/g, compared to 28.5 mg/g here after almost one year. Birds housed on wire are expected to have higher feather lipid concentrations than those kept on litter, due to the association with dustbathing. In the absence of a suitable (friable) substrate, such as wood shavings, birds cannot remove excess oil from the plumage by tossing litter into the plumage, which then absorbs oil. The presence of dustbathing substrate is seemingly more important in feather lipid concentration than preen gland output. Hens that had their preen glands removed at 22 weeks of age yielded no lower concentration of feather lipid (mg per g feathers) 20 weeks after the operation compared to feathers from hens that remained with their preen glands intact (Nørgaard-Nielsen and Vestergaard, 1981). It may be that sebaceous secretions from the skin produce enough lipid to maintain similar lipid levels to those hens in which their preen glands still remain intact.

5.4 Experiments 1, 3, 5b, 6b, 6c - Preen oil composition

5.4.1 Introduction

Differences in preen oil components (either the fatty acid or alcohol portions of wax esters) have been compared between chickens of different ages (Kolattukudy and Sawaya, 1974), between different diets (Apandi and Edwards, 1964), and between different species (Jacob and Hoerschelmann, 1985). Changes in circulating levels of sex hormones in birds, induced either artificially (Manna *et al*, 1983; Abalain *et al*, 1984) or naturally due to season (Kolattukudy *et al*, 1987), have also been shown to influence preen oil composition. Some previous studies have suggested that lipid derived directly from the preen gland is unlike that extracted from feathers, due to compositional differences in wax alcohols (Bolliger and Gross, 1958; Bolliger and Varga, 1960) and lipid shape (Ishida *et al*, 1973). It may be that these compositional differences affect the odour of the lipid and hence also that of the plumage to which it is applied. Since chicken behaviour can be affected by olfactory cues (Jones and Faure, 1982), this has been raised as a possible factor in inducing birds to feather peck at another's plumage (McKeegan and Savory, 2001). The purpose of the analyses

presented here was to explore if fatty acid components of wax esters differ with bird age, feather pecked status, or source of oil (preen gland or feathers).

5.4.2 Materials and Methods

The details on preen oil collection methods and processing are given in Chapter 2 section 2.6. Preen oil samples were collected from birds that were part of other experiments described in this or previous chapters. Comparisons were made between different bird ages (Experiment 1, prepared after freezing for 6 – 8 weeks at -20° C, and Experiment 6c, prepared when fresh), different beak treatments (beak trimmed vs not beak trimmed, Experiment 1), different feather pecking and/or feather pecked status (Experiments 3 and 6b, frozen for 1.5 and 5 weeks, respectively), and different lipid source (Experiment 5b, preen gland vs feathers, both frozen for up to 16 weeks). In glands from Experiment 6b (from 71-week-old hens), lipid could not be squeezed out of the papilla due to its solid state, thus a lump of wax was removed via an incision in one lobe of the gland. Lipid was sourced direct from the preen gland (either by collection in live birds using capillary tubes, or from dissected glands), or from feathers (in Experiment 5b). With bird age comparisons (Experiments 1 and 6c), samples were prepared by age. With all other comparisons, samples from each treatment were prepared together on any one day. All data were +1 log transformed (due to zero figures) and analysed by one-way or two-way ANOVA, with day of sample processing and side of house used as blocking factors. Mean proportions (%) of fatty acids per treatment ± standard error are presented in the original scale.

5.4.3 Results

There were 19 fatty acids identified over all samples, of which three were not affected by treatment. The mean proportions of the fatty acids ± standard error are shown in Table 5.4, listed by increasing retention time. Fifteen fatty acids were identified by retention time from GC-MS (see Chapter 2 section 2.6) and are therefore listed by the number of carbon atoms (C) and number of double bonds (listed after a colon) that they contain. Four fatty acids were unidentifiable, and these are listed by their retention time (RT) of peaks only (in minutes) for this GC column. Some were recorded at such low

concentrations that statistical comparisons could not be made (indicated by a dash mark in the P value row). An example of a gas chromatogram is given in Appendix F.

Preen gland or feather lipid was predominantly made up of C10:0, C12:0, C14:0, C16:0, C17:0, C18:0, C19:0, and C20:0, which constituted between 85.3% (Experiment 1) to 95.9 % (Experiment 3) of the total content in all treatment groups. In both Experiments 1 and 6c, age significantly affected relative proportions of fatty acids in preen oil (by two-way and one-way ANOVA, respectively). Overall proportions of each fatty acid in the range C13:0 – C18:0 were similar between these two experiments (1 and 6c). Proportions of unknown fatty acids RT 11.9 and RT 12.3 decreased with bird age over 10 – 20 weeks in Experiment 1, and also decreased over 15 – 30 weeks in Experiment 6c, however proportions at ages 15 and 20 weeks were not the same in either of these unknown fatty acids between the two experiments (Experiment 1: 0.00 % for RT 11.9 and RT 12.3, at 15 and 20 weeks of age; Experiment 6c: 0.30 % and 0.28 % for RT 11.9, 0.19 % and 0.18 % for RT 12.3, at 15 and 20 weeks of age, respectively). Other fatty acids that changed significantly with age did not follow the same trend between Experiments 1 and 6c. There were no significant effects on proportions of fatty acids of either beak treatment (BT vs NBT) or an interaction between age and beak treatment, (by two-way ANOVA, Experiment 1).

Table 5.4 *Overall mean percentages of individual fatty acids (as a proportion of total fatty acids), ±SE, and their significance (P value) by treatment per experiment. All fatty acids are listed in order of retention times, from shortest to longest. Known fatty acids are identified by number of carbon atoms (C), and number of double bonds, after a colon (where possible). Unknown fatty acids are listed by retention time (RT) only, (in min). Data were +1 log transformed and analysed by one-way or two-way ANOVA. Data presented here are original figures. Different superscripts within a column, within a group (separated by a line) denote significantly different.*

Exp. no.	RT 5.4	RT 6.2	C8:0	RT 11.9	RT 12.3	C10:0	C11:0	C12:0	C13:0	C14:0	C16:0	C17:0	C18:0	C18:1	C19:0	C20:0	C21:0	C22:0	C24:0
1	2.85	5.62	0.00	1.50	1.31	1.85	0.26	3.20	1.02	13.92	23.22	6.15	25.16	1.45	9.86	1.93	0.05	0.66	0.00
n = 12	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±
	1.26	2.84	0.00	0.68	0.62	0.75	0.11	0.58	0.18	1.25	1.08	0.78	1.42	0.50	0.65	0.33	0.04	0.66	0.00
P	0.003	0.399	-	<.001	0.001	0.108	0.221	0.001	0.010	0.002	0.050	<.001	0.181	0.636	0.091	0.098	-	-	-
(Age)	10^a	-	-	10^a	10^a	-	-	10^b	10^b	10^b	10^b	10^b	-	-	-	-	-	-	-
	15^b			15^b	15^b			15^a	15^a	15^a	15^a	15^a							
	20^b			20^b	20^b			20^b	20^b	20^a	20^a	20^a							
P (BT vs NBT)	0.401	0.348	-	0.847	0.929	0.858	0.903	0.693	0.672	0.779	0.292	0.549	0.389	0.502	0.291	0.844	-	-	-
P (Interaction)	0.208	0.634	-	0.961	0.992	0.999	0.840	0.932	0.221	0.951	0.483	0.705	0.359	0.729	0.534	0.550	-	-	-
6c	0.56	2.17	0.01	0.14	0.09	3.02	0.75	7.16	1.67	15.34	20.49	6.98	22.42	1.02	13.11	4.21	0.51	0.00	0.35
n = 4	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±
	0.19	1.47	0.01	0.04	0.02	0.41	0.16	0.93	0.24	1.53	0.61	0.47	1.39	0.30	1.32	0.67	0.14	0.00	0.19
P	<.001	0.935	-	<.001	<.001	0.049	<.001	<.001	<.001	<.001	0.050	0.022	0.030	0.143	<.001	0.044	0.270	-	0.007
(Age)	15^a	-	-	15^a	15^a	15^a	15^a	15^a	15^a	15^a	15^a	15^a	15^a	-	15^a	15^b	-	-	15^b
	20^b			20^a	20^a	20^{ab}	20^{ab}	20^{ab}	20^{ab}	20^{ab}	20^a	20^{ab}	20^{ab}		20^b	20^{ab}			20^a
	25^c			25^b	25^b	25^b	25^{bc}	25^b	25^b	25^b	25^{ab}	25^{bc}	25^{ab}		25^b	25^a			25^b
	30^c			30^b	30^b	30^b	30^c	30^c	30^c	30^c	30^b	30^c	30^b		30^c	30^a			30^b
3	0.60	0.54	0.00	0.03	0.02	0.16	0.00	0.36	0.09	2.79	20.86	13.59	29.94	0.35	18.67	9.53	2.20	0.27	0.00
n = 2, 5, or 6	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±	±
	0.20	0.33	0.00	0.02	0.02	0.06	0.00	0.08	0.03	0.33	1.28	0.74	0.81	0.08	0.87	0.91	0.36	0.08	0.00
P (Feather pecker and pecked status)	0.838	0.853	-	-	-	0.533	-	0.045 FP,P^a FP,NP^b NFP,P^a NFP,NP^{ab}	0.055	0.004 FP,P^{ab} FP,NP^c NFP,P^a NFP,NP^{bc}	0.130	0.171	0.221	0.384	0.024 FP,P^a FP,NP^a NFP,P^b NFP,NP^a	0.113	0.687	0.551	-

Table 5.4 *(continued).*

Exp. no.	RT 5.4	RT 6.2	C8:0	RT 11.9	RT 12.3	C10:0	C11:0	C12:0	C13:0	C14:0	C16:0	C17:0	C18:0	C18:1	C19:0	C20:0	C21:0	C22:0	C24:0
3 (cont)																			
P (n = 11 or 8) (Feather pecker vs non-feather pecker)	0.677	0.370	-	-	-	0.446	-	0.792	0.777	0.471	0.068	0.674	0.266	0.806	0.070	0.570	0.825	0.973	-
P (n = 7 or 12) (Pecked vs non-pecked)	0.909	0.794	-	-	-	0.406	-	0.008 P > NP	0.033 P > NP	0.002 P > NP	0.510	0.064	0.672	0.717	0.241	0.032 P < NP	0.148	0.279	-
6b	0.00	0.30	0.00	0.15	0.14	0.37	0.05	0.62	0.17	2.19	9.86	5.29	34.94	0.47	25.86	16.40	3.03	0.18	0.00
n = 9	± 0.00	± 0.11	± 0.00	± 0.05	± 0.05	± 0.15	± 0.04	± 0.28	± 0.07	± 0.65	± 1.18	± 0.38	± 1.27	± 0.08	± 0.95	± 1.27	± 0.43	± 0.07	± 0.00
P (Pecked vs non-pecked)	-	0.866	-	0.565	0.539	0.328	-	0.236	0.198	0.408	0.436	0.073	0.728	0.894	0.606	0.566	0.840	0.275	-
5b	2.48	6.41	0.06	0.22	0.19	2.90	0.46	3.45	0.66	7.69	18.59	4.75	26.84	1.22	14.22	8.12	1.17	0.28	0.30
n = 8	± 035	± 2.72	± 0.03	± 0.03	± 0.03	± 0.50	± 0.09	± 0.62	± 0.17	± 1.26	± 1.85	± 0.31	± 1.46	± 0.35	± 2.11	± 1.02	± 0.23	± 0.04	± 0.15
P (Feather vs Preen gland)	0.113	0.785	0.663	0.699	0.257	0.039 F > PG	0.065	0.496	0.589	0.539	<.001 F > PG	0.052	0.517	<.001 F > PG	<.001 F < PG	0.040 F < PG	0.006 F < PG	0.556	0.019 F > PG

In Experiment 3, not all hens (7 per category) yielded preen oil when the gland was squeezed, and those that did required a few minutes of 'kneading' before oil could be collected in capillary tubes. Only two preen oil samples were obtained from NFP,P hens, hence there was not enough information to test interaction effects and only one-way ANOVA was used on the combined feather pecker and pecked status groups. There were few differences between groups of hens. Levels of C12:0 were significantly higher in FP,P (0.58 % ± 0.16) and NFP,P (0.67 % ± 0.25) than FP,NP (0.14 % ± 0.05), but these 3 categories did not differ from NFP,NP (0.28 % ± 0.12). C14:0 was significantly higher in NFP,P (5.5 % ± 0.1) than in NFP,NP (2.3 % ± 0.3) and FP,NP hens (1.8 % ± 0.2) but did not differ significantly from FP,P (3.6 % ± 0.6), while FP,P was greater than FP,NP, but did not differ from NFP,NP. NFP,NP did not differ significantly from FP,NP with C14:0. Levels of C19:0 were significantly lower in NFP,P (12.7 % ± 1.3) than in all other categories (FP,P 18,8 % ± 1.7, FP,NP 20.9 % ± 1.1, NFP,NP 18.3 % ± 1.3), but these 3 categories did not differ from each other.

When categories from Experiment 3 were examined separately by feather pecker status, there were no significant differences in fatty acid proportions between FP and NFP (by one-way ANOVA). However, when comparing by pecked status, proportions were significantly higher in P than NP with C12:0 (0.61 % ± 0.12 vs 0.21 % ± 0.07, respectively), C13:0 (0.17 % ± 0.05 vs 0.05 % ± 0.02), and C14:0 (4.11 % ± 0.54 vs 2.01 % ± 0.20) (all by one-way ANOVA). Levels of C20:0 were significantly higher with NP than with P (10.99 % ± 1.20 vs 7.03 % ± 0.75 respecitvely). With birds classed as feather pecked or not (P vs NP) in Experiment 6b, there were no significant differences with any fatty acid between the two groups (by one-way ANOVA).

While preparing lipid extracted from feathers and from preen glands (Experiment 5b) for GC analysis, a much stronger odour was noticed from feather lipid than from preen gland extract at the solvent evaporation phase just prior to TLC (pers. obs.) (see section 2.6). Comparisons of proportions of fatty acids recovered from feathers or preen glands revealed several significant differences in composition. Oil from feathers had higher proportions of C10:0, C16:0, C18:1, and C24:0 than did oil from the preen gland. Oil

from preen glands had higher proportions of C19:0, C20:0, and C21:0 than did that from feathers.

5.4.4 Conclusions

The fatty acid profiles of preen oil derived from the preen gland examined in treatments used here are similar to that reported by Haahti *et al* (1964), who found that preen glands contained fatty acids ranging from C10:0 to C20:0. C18:2 (linoleic acid) was not apparent in preen oil analysed here. This may be because C18:2 is derived directly from the diet (Moss and Lough, 1968), whereas fatty acids in preen oil are mainly synthesised in the gland (Stevens, 1996). Although preen oil in some tinamous species contained low proportions (0.1 – 14.3 %) of C18:2 (Jacob and Hoerschelmann, 1985), lipids extracted from chicken, turkey, and duck preen glands were reported to be made up of only small proportions of C18:2, ranging from 0 – 1.37 % (Apandi and Edwards, 1964).

Changes in preen oil composition which occur with bird age are most likely related to sexual maturity and therefore hormonal state in the growing fowl. Layer pullets experience an increase in certain hormones (luteinising hormone, oestrogen, progesterone) around the onset of lay. In both studies that examined age effects (Experiments 1 and 6c), the onset of lay occurred between the 15 and 20 weeks of age sampling periods. Kolattukudy and Sawaya (1974) found that, in preen oil taken from chickens approximately once a month from 3 to 13 months of age, the proportion of C24 diols (the alcohol portion of the lipid, which was not measured here) decreased while the proportion of C23 diols increased with bird age. In ducks, analysis of preen oil from adult females at the peak of the breeding season showed a greater proportion of unbranched (i.e. straight chain) fatty acids compared to female ducklings treated with estradiol injections or without, indicating a hormonal effect on preen oil composition (Jacob *et al*, 1979). Increases in circulating levels of hormones around the onset of lay may be responsible for changes in fatty acid concentrations with bird age shown here. It cannot be ruled out, however, that differences between different ages may have been affected by processing: with Experiments 1 and 6c, on any day of GC preparation, only samples from one age were prepared. Age-related effects may also be having an impact on the consistency of preen oil, since it was noticed that wax had to be removed in an

entire lump from the glands of Experiment 6b birds (age 71 weeks), regardless of plumage status, and that preen oil was difficult to collect from live birds using the capillary tube method in Experiment 3, where birds were also 71 weeks of age.

Preen oil composition may be related to feather status. With birds classified as feather peckers or not, based on feather pecks given while in group cages, and as feather pecked or not, based on feather damage scores (see Chapter 4 section 4.3.2), there were 3 significant difference (with C12:0, C14:0 and C19:0) amongst the 4 categories, however there was no discernible trend in the differences in composition associated with feather status. Although no differences were seen in any of the fatty acids measured with birds classed as feather pecked or non-feather pecked only in Experiment 6b, birds classed in this way in Experiment 3 revealed 4 significant differences (with C12:0, C13:0, and C14:0, all P > NP, and with C20:0, NP > P). There were no significant differences between groups in Experiment 3 when classed as just feather peckers or non-feather peckers. This suggests that whether or not a bird is a feather pecker does not depend on differences in its preen oil composition, but whether or not a bird is targeted for feather pecking may be. It would be interesting to explore if differences exist between feather pecked categories with lipid extracted directly from feathers (instead of from the preen gland), as differences in sebaceous secretions from the skin might also contribute to differences in plumage odour and/or taste.

When comparing the fatty acid concentrations of oil from feathers to that taken directly from preen glands (Experiment 5b), there were distinct differences among 7 of the fatty acids recorded (C10:0, C16:0, C18:1, C19:0, C20:0, C21:0 and C24:0), with values often doubling between the two groups. After extracting lipids and isolating alcohol fractions from feathers, Bolliger and Gross (1958) determined that these were compositionally different to lipids from preen glands, due to the absence of octadecanol (C_{18}) in feather extracts, which is the main alcohol component of preen gland lipid. In another study, methylated fatty acids from feathers yielded stearic (C18:0) and palmitic (C16:0) fatty acids (Bolliger and Varga, 1960), the latter of which was found in significantly greater proportions in lipids extracted from feathers compared to those obtained from preen glands in this study (Experiment 5b). Zeman (1988) separated feather and preen gland lipid by TLC and found that feather lipid was made up of

monoesters, diesters, triesters, and triacylglycerides, whereas preen gland lipid was almost solely composed of diester and triester fractions, indicating that the two lipids were different compounds. Some authors suggest that feather lipids are derived as a by-product of feather keratinization (Bolliger and Gross, 1958; Bolliger and Varga, 1960; Bolliger and Varga, 1961). More convincing proof based on histological examination indicates that the epidermis produces sebaceous secretions (including the epidermal layer that lines feather follicles), and should therefore be considered a gland itself (Lucas and Stettenheim, 1972a; Ishida *et al*, 1973; King and McLelland, 1984; Stevens, 1996). Epidermal secretions could explain why some fatty acids vary in concentration between extracts from feathers to those from preen glands. Although preen oil is distributed over the plumage during preening (and is thus present in lipid extracted from the plumage), the skin may be producing enough lipid independently to affect the fatty acids seen in GC analysis. This supplementary lipid production could partly explain why the plumage of preen gland extirpated ducklings that have not been allowed to apply preen oil prior to extirpation have been shown to maintain waterproofing properties for at least 1 week post removal (Fabricius, 1959). The observed difference in odour between lipid extracted from feathers with that from preen glands (pers. obs.) may be attributed to differences in lipid composition, since other (non-lipid) molecules had been filtered and washed off prior to the difference in odour being noticed. This odour could conceivably affect plumage taste and therefore its attractiveness to other birds as a pecking inducer (McKeegan and Savory, 2001)

5.5 Discussion

These findings show that floor substrate had no effect on preen gland weight and dimensions that could not be attributed to bird weight. However, the amount of lipid extractable from feathers using petroleum ether was affected by the presence of wire or litter floor substrates. This suggests that it is not preen gland size that affects feather lipid levels, but the presence or absence of a dustbathing substrate. The absence of litter in wire floor environments results in non-functional (sham or vacuum) dustbathing, and hence an inability to remove stale lipid from the plumage.

Bird age affected all parameters measured here. Mean preen gland weight, dimensions, and zone areas, and feather lipid levels all increased with age, and levels of some fatty acids changed significantly. Although feather lipid levels remained fairly constant after 5 weeks in Experiment 1, levels here may have increased with age had the experiment not terminated at 20 weeks. The greater concentration of feather lipid at age 30 weeks in Experiment 6c cannot be accounted for by a change in relative preen gland size (which remained fairly constant after age 5 weeks). It may be that presumed sebaceous secretions from the skin increase at this age. Other factors that might affect feather lipid concentration include preen oil application, plumage area, and time spent dustbathing.

A bird's feather pecker or pecked status is seemingly unrelated to either relative preen gland size and dimensions, or (with feather pecker status) composition of preen oil. Despite distinct differences in plumage damage between groups in Experiment 6b, no differences were found in either gross morphology of preen glands or preen oil composition. However, in Experiment 3, preen oil composition may have affected whether or not a bird was pecked, but did not seem to influence if a bird was a feather pecker or not. It may be that such differences are affecting the odour or taste of preen oil (McKeegan and Savory, 2001), and thus the plumage of some hens may be more attractive for pecking and eating than others. Differences between birds in sebaceous secretion composition that may affect feather odour and/or taste, however, cannot be ruled out.

Collectively, these experiments indicate that preen gland function is strongly affected by bird age and therefore development. Apart from increased feather lipid levels on wire floors in one study, it does not appear to be related to external influences such as floor substrate. Preen oil composition may be affecting whether or not a bird is feather pecked. There is evidence here and in published work that the preen gland is not the only source of lipid on the plumage.

Chapter 6

General Discussion

The aims of this project were to assess how bird age, beak trimming, and various external influences affect preening. The function of the preen gland, including gross morphology and histology, feather lipid quantity and preen oil composition, was also investigated, since this physiological aspect may well interact with preening behaviour.

The proportion of time spent preening, across all behaviour studies, ranged from about 2 % (Experiment 5a) to almost 11 % (Experiment 1). The experiments which revealed the highest proportions of time preening (almost 11% and 9 % in Experiments 1 and 3, respectively) employed instantaneous recording methods, which are less accurate than continuous recording. Also, Experiment 1 looked at birds from day old, whereas all other studies examined mature (laying) hens. Experiment 2 looked at hen behaviour over only 3 x 20 min sampling periods, and though no differences were detectable, frustration of feeding in the 4 and 8 h deprivation treatments may have caused the overall time spent preening to be relatively high (about 7 %) compared with other experiments using continuous recording. It is probably more accurate, therefore, to say that laying hens spend 2 – 3 % of their time preening, based on studies that continuously recorded preening over entire light cycles (Experiments 5a and 5b).

In Experiment 1, beak trimmed birds spent significantly more time at the preen gland when both standing and sitting than did non-beak trimmed birds (Table 3.3), but the concentration of feather lipid did not differ with beak treatment (section 5.3.3). Presumably this was because, due to the alteration in beak morphology, beak trimmed birds took longer to collect the same amount of preen oil from the gland than did intact birds. Despite feather lipid concentration being highest at 5 weeks of age, preen gland use was greatest when birds were 6/7 weeks old while standing, and 8/9 weeks old while sitting.

Another factor that affects the concentraiton of feather lipid is functional dustbathing. Van Liere and Bokma (1987) have shown that feather lipid concentration increased

when hens were deprived of sand in which to dustbathe for 33 days, but returned to pre-deprivation levels once sand was accessible again. Previously, Savory and Mann (1997) showed that time spent dustbathing increased progressively after 12 weeks in Hisex birds (a modern laying strain) studied to 24 weeks. However, in this study (Experiment 1), in which loose litter was always present, there were no significant differences in time spent dustbathing over the age range observed (0 – 19 weeks of age), nor were there significant differences in feather lipid concentration with bird age, apart from at 5 weeks of age, when presumed pulp from the growing feather calamus was also eluted. Similarly, there were no differences in proportions of times spent at the preen gland or in (functional or non-functional) dustbathing between floor treatments in Experiments 5a and 5b, and there were also no differences in relative preen gland weights with floor treatments in Experiments 5b or 6a. However, feather lipid concentration was significantly higher in birds housed on wire for almost a year (Experiment 5a), suggesting that presence or absence of a suitable dusbathing substrate is largely responsible for differences in feather lipid levels. This is because birds housed on litter can perform functional dustbathing, in which litter penetrates the plumage and removes a build up of oil. Although birds on wire carry out (sham or vacuum) dustbathing movements, no litter is tossed into the plumage and therefore lipid remains on feathers, resulting in more lipid extracted per g feathers.

With feather pecking categories in Experiment 3, there were no differences in times spent at the preen gland, but there were 4 significant differences with pecked or not pecked status, and 3 significant differences between the combined feather pecker/non-feather pecker and pecked/non-pecked statuses in preen oil composition. There were no differences in preen gland measurements (weight, weight as a percentage of body weight, dimensions, or preen oil composition) between pecked and non-pecked end-of-lay hens from Experiment 6b. These findings suggest that neither preen gland use nor gross morphology are causally linked with feather pecking, but that preen oil composition may be. It has been suggested that preen oil may act as an attractant or reinforcer in feather pecking (McKeegan and Savory, 2001), which may be related to its odour. McKeegan and Savory (2001) showed that the propensity for known feather and non-feather pecker hens to peck at, manipulate, and eat unwashed feathers was

significantly greater than with washed feathers (which presumably removed preen and skin oil), suggesting that substances present on unwashed feathers makes them an attractive pecking substrate. Other work also indicates an association between feather pecking and preening, since birds that received severe feather pecks were preening more often than expected (Keeling, 1995), or high receivers of feather pulls tended to show more preening behaviour (Savory and Griffiths, 1997). Further information could be gathered by presenting feathers from feather pecked and non-feather pecked hens to known feather peckers, and observing the number of feathers pecked at or eaten from each group. Feathers may be selected by feather pecking hens for odour and/or taste differences (caused by differences in preen oil composition) detectable by them between pecked and non-feather pecked birds' plumage. However, rather than preen oil, it may be that the composition of sebaceous secretions from the skin differ between feather peckers and/or feather pecked hens, giving some birds an odour and/or taste that attracts feather pecking. Birds in cage systems may be more likely to be feather pecked than those housed with access to litter, because the lack of a friable dustbathing substrate results in higher feather lipid concentrations (as in Experiment 5a) which may make their feathers more attractive than birds' feathers with lower concentrations of lipid.

Preen glands from hens housed in cages on wire for one year (Experiment 6b, bird age 71 weeks) or in pens on litter for 30 weeks (Experiment 6c, bird age 30 weeks) were sometimes firm to the touch. When collecting preen oil for GC analysis, the lipid from all glands in Experiment 6b (whether assessed as firm or not) had to be removed as a lump from the gland because of its solid state, while collecting preen oil in capillary tubes from live birds in Experiment 3 (where birds were also age 71 weeks) was difficult and sometimes impossible. It may be that, regardless of the presence of litter for dustbathing, the preen gland becomes congested with solidified lipid as the bird ages, which may have implications for preen oil application and plumage condition. Birds of all ages (Experiment 1), and on both wire and litter substrates (Experiments 5a and 5b) showed at least some time spent at the preen gland and in dustbathing behaviour. However, if some or all birds' glands eventually become congested, it may be that older birds have difficulty in collecting oil from the gland (although earlier applications and possible continual secretion from the skin may serve to keep oil present

on plumage). Therefore, it may be the lack of fresh preen oil, and/or the presence of stale oil (due to absence of a friable dustbathing substrate), that makes feathers attractive to pecking. This may contribute to the apparent increase in feather loss seen in many laying hens, in both cage and litter floor systems, towards the end of lay.

Due to lack of time, some interesting areas of study were never pursued in this 3-year project. For example, the sequence of body sites targeted during preen bouts was not examined. Van Liere *et al* (1991) found that, after hens had collected oil from the preen gland, they preened the breast first, followed by the wings and then the flanks. Sandwich terns and common terns were observed to begin preening activity after bathing with headshakes or preening the breast, followed by preening the wings (van Iersel and Bol, 1958). Preen gland extirpation and its effects on preening behaviour, dustbathing, and the propensity to be feather pecked all warrant further investigation, although the ethical implications regarding possible suffering (surgical procedure, post-operative pain, inability to apply preen oil) for the birds involved would have to be seriously considered. Examining birds with and without preen glands could indicate the importance (or not) of preen oil in stimulating preening behaviour, maintaining good plumage condition, dustbathing motivation, and likelihood of being feather pecked. Such an experiment could also contribute to information about the role of skin lipid and its production.

Another topic that could have been investigated is a possible effect of red mites (*Dermanyssus gallinae*) on preening behaviour in laying hens. Chickens infested with Mallophaga lice have been shown to significantly increase preening behaviour compared with uninfested groups (Brown, 1974). Although it may be difficult to control red mite populations, the behaviour of groups of hens infested with red mites could be compared to those not infested, to examine differences in preening and dustbathing behaviour. Since inhibition of self-grooming behaviour has been shown to increase parasitic load in mice (Murray 1961 and 1987, as cited in Hart, 1992), the effects of beak trimming could be tested in groups of hens infested with red mites. Number of mites per beak treatment group could be estimated by isolating birds in small chambers at night (when mites are more likely to attach themselves to birds, due to their nocturnal behaviour), and then counting red mites that have dropped off by day.

If red mites are attracted to, or feed on, lipid on the plumage, the ability to carry out functional dustbathing (in a friable substrate) might reduce a bird's attractiveness to the mites. Kirkwood (1971) found that although red mites fed *in vitro* would ingest all types of blood offered (quail, chicken, sheep, calf, pig, and rabbit blood), they would only feed through (washed) quail or chick skin, as opposed to synthetic membranes or mammal skin. However, Zeman (1988) found that red mites preferred to feed on chicken blood *in vitro* through (in order of preference): Parafilm impregnated with feather extract, unwashed chicken skin, and Parafilm impregnated with preen gland secretion, compared to washed skin or Parafilm impregnated with hydrolysed feather extract, methylesters, or nothing. Offering mites Parafilm impregnated with various fractions of feather and preen gland lipid extract (obtained by TLC or column chromatography) revealed that the diester (and possibly triester) fractions were most likely to illicit feeding (at least 26 % of mites fed through Parafilm impregnated with these, as opposed to at most 17 % with other fractions) (Zeman, 1988). But was the odour of unwashed skin or diesters from feathers or preen glands the attractant, or might the red mites also be feeding on skin and/or preen lipids? To answer the former question, mites could be tested in a Y-shaped chamber, with various odours blown down 2 of the arms of the 'Y' towards mites placed at the end of the third arm. Odours used could be blood, preen oil, feathers and skin (the last 2 in both washed and unwashed form). The number of mites that migrate towards each odour would indicate that odour's attractiveness. Giving colonies of red mites *in vitro* feeding choices between filter paper saturated with blood, preen oil, both, or none might determine what red mites ingest. TLC or GC analysis could be carried out on red mites to determine what they had eaten and compared by TLC or GC to unfed mites, or compared to blood and preen oil profiles.

This project has demonstrated that preening is influenced by bird age and can be expressed synchronously within groups, but is not greatly affected by external influences such as (minor) beak trimming, frustration of feeding, feather pecker or pecked status, or floor substrate. Although occupying only a small proportion of the birds daily time budget, preening fulfils an important role in feather maintenance and is closely linked to dustbathing. The presence of preen and possible skin oil on plumage

may affect thermoregulation, feather eating and pecking. Although composition of preen oil does not appear to differ greatly with feather pecking or pecked status, older birds may tend to have congested preen glands, possibly leading to reduced efficiency of preen oil extraction and altered plumage odour, which may attract pecking and increase feather loss.

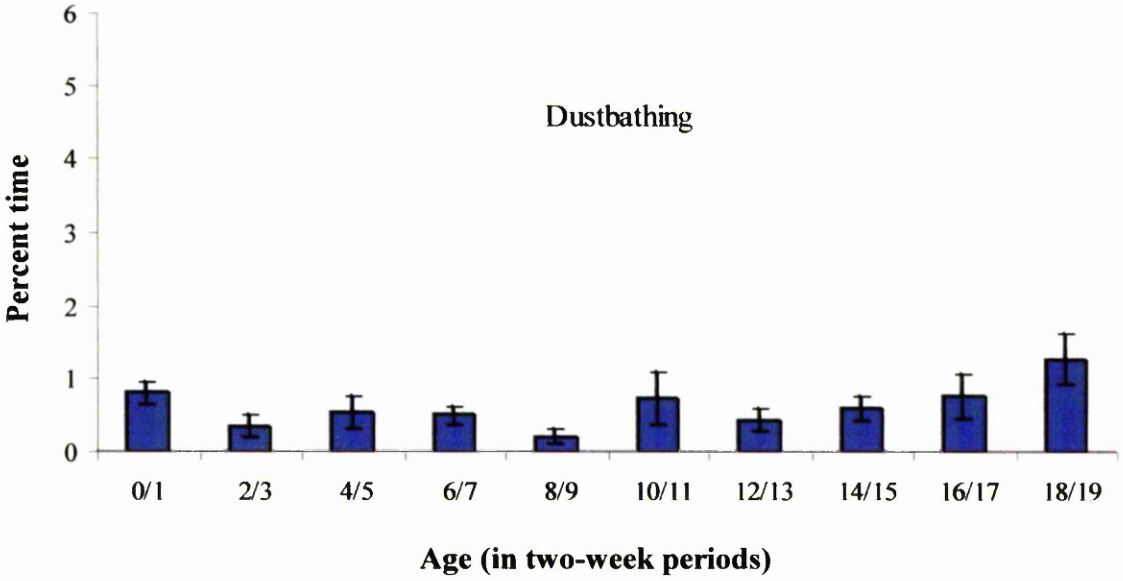
Appendix A

Explanation of data collected in each study.

Experiment	Behaviour observations		Bout analysis		Feather lipid concentration	Feather or preen gland lipid composition	Preen gland		Beak lengths
	remote	direct	defined	applied			morphology	histology	
Pilot	✓								
1 & 4		✓			✓	✓			✓
2	✓			✓					✓
3		✓				✓			
5a	✓		✓	✓	✓				✓
5b	✓				✓	✓	✓		✓
6a							✓		
6b						✓	✓		
6c					✓	✓	✓	✓	

Appendix B

Dustbathing behaviour in layer pullets from 0 to 19 weeks of age.



Appendix C

Ethogram of behaviours and symbols used in scan sampling, using Keybehaviour software.

Behaviour	Symbol	Behaviour	Symbol
Preening while standing:		sitting only	-
breast	/b	standing only	/
back	/k	walking	w
wing	/w	feeder directed	f
flank	/f	drinker directed	d
tail	/t	wing flapping	wf
preen gland	/g	shaking (head, body, tail)	k
Preening while sitting:		head scratching	hs
breast	-b	litter directed	ld
back	-k	spot pecking	pp
wing	-w	non aggressive pecking	np
flank	-f	aggressive pecking	ap
tail	-t	bill wiping	bw
preen gland	-g	stretching (wing, leg)	s
		dustbathing	db

Appendix D

Original data file

Example of an original scan sampling session (15 min x 8 birds) from Experiment 1, pen 10, age 16 weeks, using notation described in Appendix C.

	Bird							
Time (min)	**white**	**yellow**	**orange**	**red**	**ltblue**	**green**	**purple**	**black**
1	w	/	f	/	ld	f	/	/
2	w	w	w	/	/	ld	w	f
3	s	/	/	f	w	ld	/	/
4	f	/	/	w	k	f	/w	/
5	hs	ld	/	ld	/	/w	/b	/
6	/k	/	w	ld	/f	/b	/b	/
7	ld	/b	-w	/	/k	/b	/w	/
8	/k	ld	-	w	/w	/b	/f	-
9	/b	f	-	ld	/w	/t	/f	/b
10	/w	w	-	/	/b	/	/w	/
11	/f	w	-	f	/k	/b	/w	/f
12	/f	/	/w	f	/b	/k	/b	/
13	ld	-	-	-	-	-	-k	/
14	/k	/f	w	/	-	-	/b	/b
15	/k	/f	/b	/	-	/w	/b	/

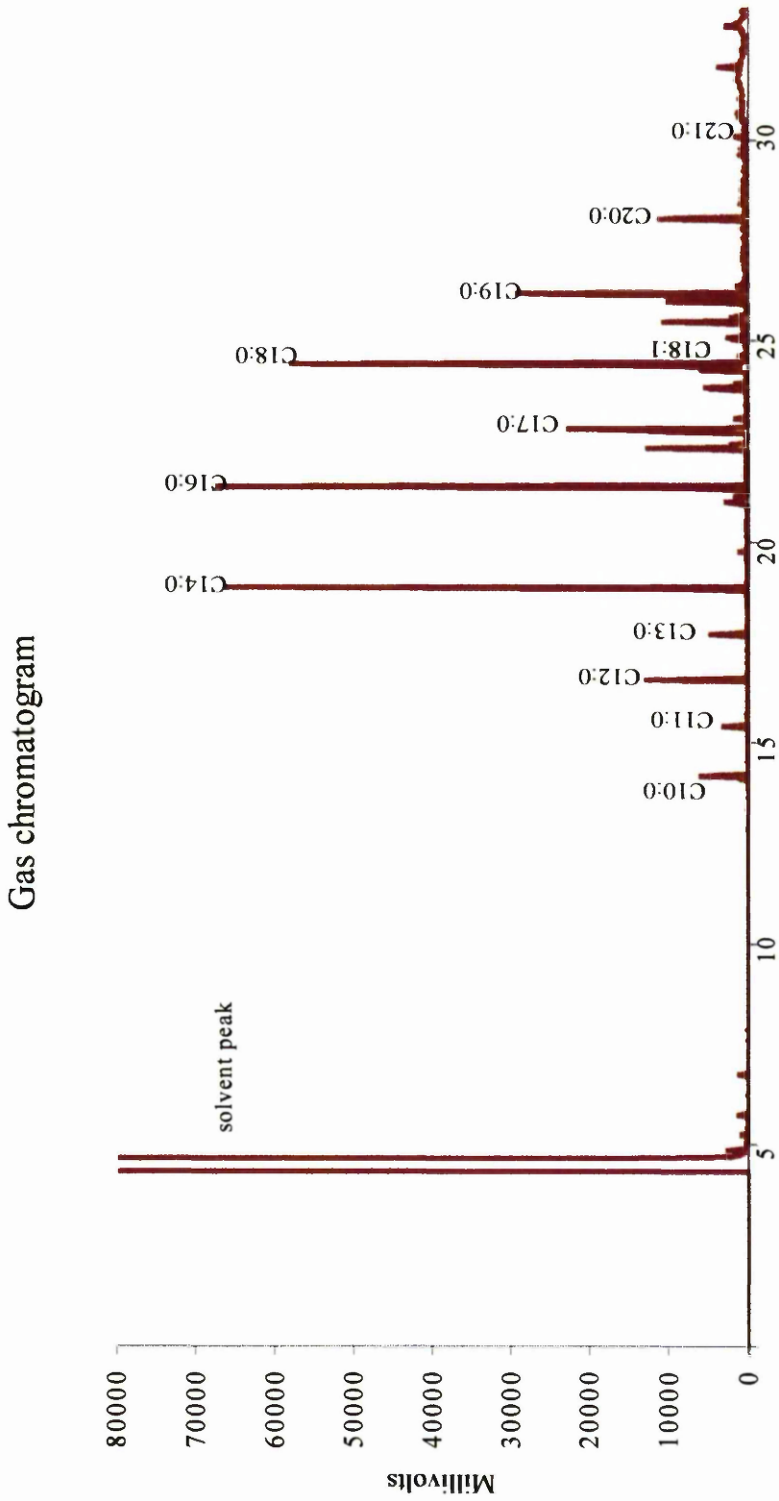
Appendix E

Allelomimetic analysis

Scan sampling session shown in Appendix D, converted to show preening only data, where applicable. In this example, 44 events of preening occurred out of a possible 120 (8 birds x 15 min), giving a total of 36.7% preening in this session.

	Bird							
Time (min)	**white**	**yellow**	**orange**	**red**	**ltblue**	**green**	**purple**	**black**
1	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0
4	0	0	0	0	0	0	1	0
5	0	0	0	0	0	1	1	0
6	1	0	0	0	1	1	1	0
7	0	1	1	0	1	1	1	0
8	1	0	0	0	1	1	1	0
9	1	0	0	0	1	1	1	1
10	1	0	0	0	1	0	1	0
11	1	0	0	0	1	1	1	1
12	1	0	1	0	1	1	1	0
13	0	0	0	0	0	0	1	0
14	1	1	0	0	0	0	1	1
15	1	1	1	0	0	1	1	0

Appendix F



Gas chromatogram of methylated fatty acids of preen oil taken from a hen age 20 weeks, Experiment 1.

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