

The effect of diet change on the behaviour of layer pullets

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Abstract

Frequent diet change has been identified as a risk factor for feather pecking in commercial flocks but the mechanism underlying this association is not known. In this experiment we simulated a commercial change of diet between high quality (HQ) 19% protein, and low quality (LQ) 15% protein, diets. Twelve pairs of birds were fed both diets simultaneously for 38 days to determine whether clear preferences for the diets existed. A further 12 triplets of birds were fed either HQ or LQ diets for 38 days to examine any absolute effects of quality on behaviour. The remaining triplets received, on day 29, either a LQ to HQ diet change ($n = 12$ groups) or an HQ to LQ diet change ($n = 12$ groups). Half of the groups in these diet change treatments received oregano oil as a potential 'masking' agent to disguise the diet change. No dietary preferences were detected and there were no absolute effects of diet on behaviour. Diet change provoked significant increases in beak-related activity. Specifically, affiliative pecking (allo-preening directed towards comb or beak) was increased after diet change. Masking the diet reduced the effects of diet change. Injurious pecking remained at low levels throughout the experiment and was not affected by diet change, but the relationship between affiliative pecking and subsequent injurious pecking requires further investigation.

Keywords: animal welfare, cannibalism, chicken, diet, feather pecking, laying hen

Introduction

Feather and vent pecking are serious welfare and economic problems in laying hens. Evidence suggests that feather pecking is a consequence of redirected foraging behaviour (Blokhuis 1986) and is most likely to occur when suitable foraging substrates are not easily accessible or when low fibre diets are fed (Huber-Eicher & Wechsler 1997; Aerni *et al* 2000). In some situations birds may even consume feathers as an alternative source of fibre (Harlander-Matauschek *et al* 2006). Feather pecking is painful to the recipient (Gentle & Hunter 1990) and both feather pecking and vent pecking can lead to cannibalism (McAdie & Keeling 2000; Pöetzch *et al* 2001), which is of particular concern in non-caged housed hens. As the European Community ban on the use of unenriched cages approaches (EC 1999), the need for research on the multifactorial causes of injurious pecking becomes more pressing. An epidemiological study of non-caged hens in the UK identified diet change as a risk factor for both feather and vent pecking (Green *et al* 2000; Pöetzch *et al* 2001) but the mechanisms underlying this risk are unclear. Recently, we demonstrated in a controlled experimental setting that young layer chicks became more active, and performed more furniture pecking and overall pecking following a diet change from a preferred to a less-preferred feed (flavoured with orange oil) (Dixon *et al* 2006). In contrast, changing

the diet from a less-preferred feed to a more-preferred feed resulted in few behavioural changes.

The aim of the current experiment was to examine whether adult laying hens exposed to a more commercial type of diet change would exhibit similar changes in activity and pecking behaviour. Commercial adult layers are subject to phase feeding. As they mature, feed intake increases whilst egg production slows and it is economical to reduce the protein being fed (Gleaves 1989; Leeson & Summers 1997). Typically, three diets are fed during the lay cycle, the protein content reducing by 1 to 2% each time. Two diet formulations were devised for our experiment that differed mainly in protein content: a 'high quality diet' (HQ) contained 19% crude protein and a 'low quality diet' (LQ) contained 15% crude protein. As in commercial feeds, the protein to energy ratio was kept as constant as possible between the diets. The difference between these two diets resembles an exaggerated version of a commercial-type diet change. A minimum of 15% protein was chosen to avoid any effects of protein deficiency *per se* on injurious pecking behaviour (Hughes & Duncan 1972). Protein contents above 15.2% are sufficient to avoid deficiency effects on pecking across several different layer strains (Ambrosen & Peterson 1997).

An additional factor investigated in this experiment was the use of a flavouring agent as it has been suggested this may mask the effects of diet change (Leeson & Summers 1997).

Table 1 Nutritional composition of HQ (high quality) and LQ (low quality) diets.

Diet	Oil (%)	Protein (%)	Fibre (%)	Ash (%)	Methionine (%)	Moisture (%)
HQ	5.57	19.00	2.47	12.59	0.45	11.15
LQ	3.42	15.00	2.35	12.42	0.34	11.85

Table 2 Diets fed to treatment groups during Familiarity and Treatment Periods (O signifies feed supplemented with 75mg kg⁻¹ oregano oil).

Treatment group	Familiarity phase	Treatment phase
Control C1	LQ	LQ
Control C2	HQ	HQ
Diet change DC1	LQ	HQ
Diet change DC2	HQ	LQ
Diet change DC3	LQO	HQO
Diet change DC4	HQO	LQO
Preference test P	LQ/HQ choice	LQ/HQ choice

The effects of familiar flavours on dietary neophobia have been investigated in domestic chicks (Jones 2000) and in other species (Cheney & Miller 1997; Launchbaugh *et al* 1997). Chicks had a shorter latency to peck at a novel textured food and ate more of it in the presence of a familiar odourant (Jones 2000). Our experiment investigated the use of oregano oil as a 'masking' agent during dietary change. Oregano oil has been suggested as a supplement to poultry feed since the use of growth promoting antimicrobials has been restricted, because it shows some *in vitro* antimicrobial effects (Cross *et al* 2002).

It is becoming apparent that relationships between different 'types' of inter-bird pecking in chickens are not straightforward. Mild feather pecking, severe feather pecking and vent pecking may share some risk factors but the occurrence of one does not necessarily predict the occurrence of the other (Rodenburg *et al* 2003; Newberry *et al* 2007). Because the relationships between these different types of pecking are not yet fully known, we recorded and analysed all categories of inter-bird pecking separately, as well as creating new categories that combined the different types.

Materials and methods

Animals and housing

One hundred and seventy four beak-trimmed layer hens ('Hy-line', Studley, Warwickshire, UK) were purchased. They had been reared in a commercial deep litter house and had been phase fed 4 different diets (containing 20, 18.5, 18 then finally 16% crude protein) through the rearing period. They had been fed the last of these diets for the 6 weeks prior to delivery at 125 days of age. The hens were randomly assigned to pairs in six pens and triplets in 54 pens. Pairs were used for feed preference tests to minimise animal numbers, whilst avoiding social isolation.

Triplets were used for all other tests to increase the chances of detecting bird-directed pecking, whilst maximising the number of independent groups for statistical analysis. These pens were distributed over eight separate but identical controlled-environment rooms. Three rooms contained seven pens and five rooms contained eight pens. Each pen was a cardboard box (0.75 × 1.00 × 0.75 m; length × width × height) containing a 0.1 m depth of wood shavings as litter, a plastic nest box (0.30 × 0.30 × 0.30 m), with a doorway (0.15 × 0.15 m; length × breadth) cut into the front face, a 0.4 m wooden perch, and a red bell drinker providing *ad libitum* access to water.

Ad libitum food was provided in either one (bird triplets) or two (bird pairs) raised galvanised metal troughs (0.306 × 0.070 × 0.0122 m) with wire lids. Troughs were placed in the front left corner of each pen, and the two troughs for paired birds were arranged in an 'L' configuration. The temperature in each room was maintained between 13 and 24°C. Light was provided by four 100 W incandescent bulbs in each corner of each room. This provided an average of 33 lux near the floor of each pen. The birds were initially given a light:dark cycle 12 h:12 h, with the light period commencing at 0600h each morning. The length of the light period was increased by 30 min, 17, 20, 23 and 27 days after the birds arrived. Routine husbandry was carried out in the morning.

Procedure

There were seven treatment groups given different exposures to the HQ and LQ diets. The composition of these diets is shown in Table 1. The six pens of pair-housed birds were assigned to the Preference Test group (Group P). Nine triplets were assigned to each of six further treatment groups according to the diet received during the 29-day Familiarity Period and a 9-day Treatment Period (Table 2). Pens were assigned to treatment groups in a pseudorandom fashion so that the treatment groups were spread as evenly as possible between rooms.

Group P was used to assess whether there were any preferences between HQ and LQ diets. Groups DC1 and DC2 were used to test whether behavioural differences resulted from diet change *per se* or from diet change in a particular direction. Control groups C1 and C2 permitted examination of any effects of being fed one particular diet rather than changing diets. Groups DC3 and DC4 enabled an assessment of the ability of the oregano oil to 'mask' the diet change.

Throughout the experiment, Group P could choose between HQ or LQ diets, presented in separate troughs, with diet position counterbalanced across pens. The consumption of each diet type by each pair of Group P birds was measured daily. Daily food consumption in the other treatment groups

Table 3 Postural category definitions for scan sampling.

Category	Definition
Standing	Bird standing anywhere other than on perch or on nest box
Moving	Bird moving (walking or running)
Perching	Bird standing on wooden perch or on top of nest box
Sitting	Bird lying or sitting down, not bearing weight on feet and not dustbathing
Dustbathing	Bird sitting or lying and performing litter tossing, feather fluffing, litter pecking or scratching, bill racking and side rubbing

Table 4 Beak activity category definitions for scan sampling.

Category	Definition
Preening	Bird preening its own feathers
Litter pecking	Bird pecking at litter on floor or accumulations of litter anywhere except inside feed trough
Food pecking	Bird pecking anywhere inside feeder
Water pecking	Bird pecking at water in drinker
Affiliative pecking	Gentle pecking at another bird's head or beak
Aggressive pecking	Hard, rapid pecks at the anterior of another bird
Particle pecking	Pecks at particles on another bird's plumage
Mild feather pecking	Gentle pecks at the plumage of another bird without pinching or pulling at feathers, not obviously directed at particles on plumage
Severe feather pecking	Vigorous pecks at the plumage of another bird or pinching and pulling at its feathers
Vent pecking	Pecking at or around the cloaca of another bird
Furniture pecking	Pecking at the box wall, nest box wall, weld mesh, perch, brick, feed trough walls or drinker walls

was measured during the last week of the Familiarity Period and throughout the Treatment Period.

On the first day of the Treatment Period (Treatment Day 1), food troughs were removed and weighed. All food was then removed and troughs refilled to a total weight of 2.1 kg with the appropriate feed, as detailed in Table 2. When the troughs were replaced in the pens, the latency to first feed peck by any of the hens in the pen was recorded.

Behavioural measures of the birds in Groups C1, C2, DC1, DC2, DC3 and DC4 were taken on Days 27 and 29 of the Familiarity Period and on Days 1, 2, 5 and 8 of the Treatment Period. The observations were made by two observers between 1300 and 1700h. Trial observations of pens had confirmed a high level of inter-observer agreement. Each room was observed at an identical time when observations were made, to facilitate longitudinal comparisons. The two observers alternated the rooms in which they made observations on successive observation days so that each pen was observed for an equal time by each observer over the entire experiment. Observers allowed a minute for birds to become accustomed to their presence before proceeding. The observations of each pen

consisted of: (i) Three minute long scan sampling (Martin & Bateson 1993) in which each of the three birds was scanned every ten seconds. The posture of the bird was recorded as one of five mutually exclusive categories and the beak activity of the bird was recorded as one of 11 mutually exclusive categories (Tables 3 and 4). And, (ii), three-minute long behaviour sampling (Martin & Bateson 1993) of all birds in the pen, in which every inter-bird peck was categorised and recorded using a handheld Psion Workabout with Observer 4.0 Software (Noldus Information Technology, Wageningen, The Netherlands). The number of pecks and the bird that performed them were recorded. Inter-bird pecks were categorised as affiliative, aggressive, particle, mild feather, severe feather or vent pecks, according to the definitions given in Table 4. In addition all furniture pecks, as defined in Table 4, were recorded.

Statistical analysis

All statistical tests were two-tailed with an alpha level of 0.05 and were conducted using SPSS Edition 11.5. Pair and triplet means were used as independent units for analyses expressed as mean \pm standard error of mean. Data were tested for normality using Kolmogorov-Smirnov and

Shapiro-Wilk tests so that the appropriate statistical tests could be deployed. Where computer power allowed, exact *P*-values are quoted for non-parametric statistical tests.

Food consumption in Group P

To assess whether Group P chickens consumed more of one diet than the other in the week prior to the Treatment Period and during it, a repeated measures ANOVA, where diet type and days were used as within subjects factors, was carried out on data from the last 15 days of the experiment.

Feed consumption in C and DC Groups

The feed consumption of the treatment groups C1, C2, DC1, DC2, DC3 and DC4 was analysed using a two-way repeated measures ANOVA on feed consumption data for the last 7 days of the Familiarity Period, and then for the entire Treatment Period (missing data for Treatment Day 6). The analysis examined the effects of planned (Familiarity Period) or implemented (Treatment Period) diet change (three levels: no diet change, diet change from LQ to HQ or from HQ to LQ) and oregano oil (two levels: absent or present).

Paired *t*-tests were used to compare the mean feed consumption from the last three days of the Familiarity Period with the mean feed consumption over the Treatment Period within each of the six treatment groups, in order to assess whether diet change affected feed consumption.

Latency to first feed peck

The latency to the first feed peck was analysed for differences between treatment groups using a Kruskal-Wallis test.

Scan samples of bird postures

Initially, differences between treatment groups were analysed either using non-parametric Kruskal-Wallis tests, with *post hoc* Mann-Whitney *U* tests or, if assumptions for parametric analysis were met, using two-way factorial ANOVAs. The ANOVAs examined the effects of diet change (three levels) and oregano oil (two levels). Data from Day 1 of the Treatment Period were analysed in this way, as were summary measures of data from the Familiarity Period and the Treatment Period. The summary measures were the sum of the data from both observations in the Familiarity Period and the sum from all four observations in the Treatment Period: these being proportional to the 'area under the curve' of a plot of frequency of the behaviour against time.

Secondly, data were analysed for differences within each group between the Familiarity and Treatment Periods. Summary measures were calculated for each behaviour during each of these periods by dividing the sum over all the observations by the number of observations to obtain a comparable mean 'rate' of each behaviour. Paired *t*-tests were used for the analysis unless data were not normally distributed, when Wilcoxon Signed Ranks tests were used instead.

Scan samples of beak activities

In addition to raw data on the 11 categories listed in Table 4, new categories were created: total non-nutritional pecking (the sum of all litter, furniture and inter-bird pecking); total injurious pecking (the sum of all mild feather pecking, severe feather pecking and vent pecking), total inter-bird pecking (the sum of all mild feather pecking, severe feather pecking, vent pecking, aggressive and affiliative pecking)

and beak inactivity (the percentage of time in which no beak activity was recorded). In addition, relative efficiency of feeding pecks was assessed, by dividing the proportion of observation time spent feeding by the amount of feed consumed. Higher values of this ratio represent less efficient feed pecking behaviour. Statistical analysis was conducted as described for bird posture.

Behaviour sampling

Behaviour sampling generated observed rates for: affiliative pecking, aggressive pecking, particle pecking, mild feather pecking, severe feather pecking, vent pecking and furniture pecking. In addition to these raw data, new categories were created: total injurious pecks (the sum of mild feather pecks, severe feather pecks and vent pecks) and total inter-bird pecks (the total injurious pecks, affiliative, aggressive and particle pecks). Statistical analysis was conducted as described for bird posture.

Results

Feed consumption

There was no significant difference in the amounts of each diet consumed by Group P during the last 14 days of the experiment (observed power = 0.149 where $\alpha = 0.05$). There were no significant effects of diet change or the presence of oregano oil on the amount of feed consumed by any of the treatment groups for the last week of the Familiarity Period (when diet change groups had been allocated but no change had yet been implemented) or for the entire Treatment Period. Neither were there any significant interactions between diet change and the presence of oregano oil on feed consumption in these groups. There were no significant differences in mean daily feed consumption between the last three days of the Familiarity Period and the entire Treatment Period for any treatment group.

Latency to first feed pecks

There were no significant differences between treatment groups in the latency to the first feed peck.

Scan samples

Postures

There were no significant differences for any of the analyses for time spent perching, dustbathing, sitting or moving. Within Treatment groups, DC1 birds spent more time standing during the Familiarity Period than they did during the Treatment Period ($t = 2.659$, $df = 8$, $P = 0.029$).

Beak activities

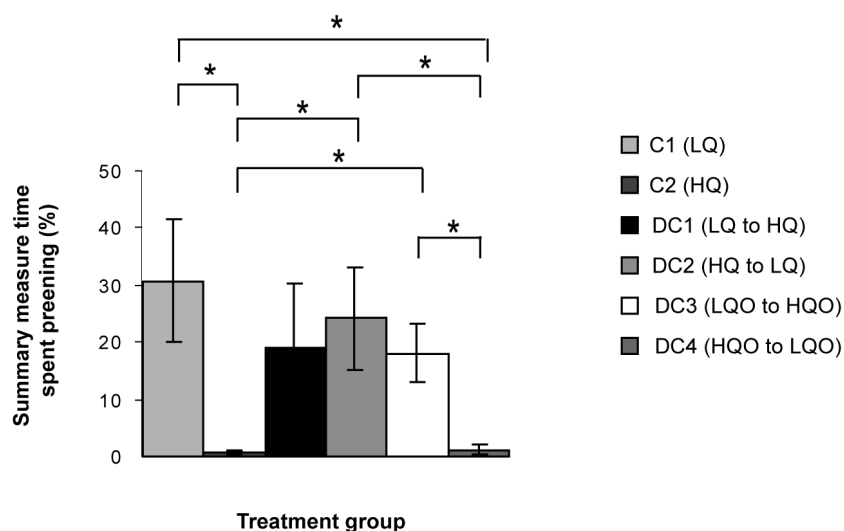
There were no significant differences for the time spent litter pecking, particle pecking, mild feather pecking, severe feather pecking, vent pecking, water pecking or furniture pecking. There were too few observations of aggressive pecking to support a statistical analysis.

Preening

There were significant differences between treatment groups in the percentages of time spent preening during the Familiarity Period (Chi-square = 21.320, $df = 5$, $P = 0.001$;

Figure 1

Time spent preening, recorded by scan sampling during the Familiarity Period.

**Figure 2**

Total non-nutritional pecking recorded by scan sampling during the Familiarity Period.

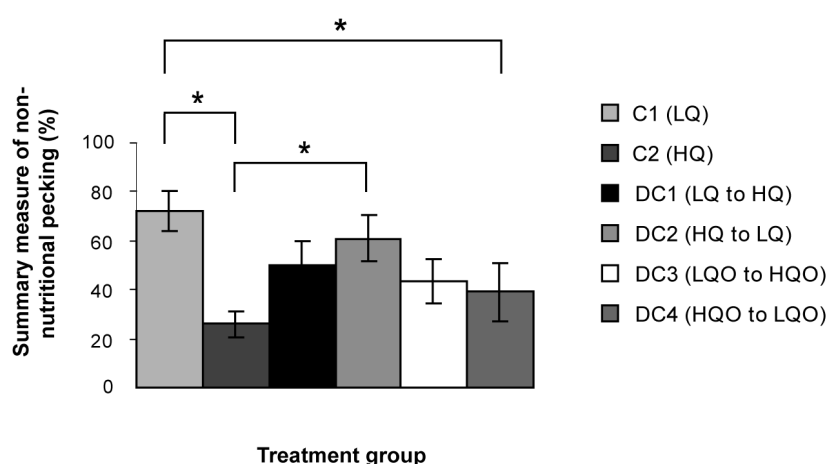


Figure 1) but not on Day 1 of the Treatment Period or across the Treatment Period as a whole.

Within treatment groups, Group C1 showed decreased preening ($t = 3.3$, $df = 8$, $P = 0.01$), whilst C2 and DC4 showed increased preening ($z = 2.52$, $n = 9$; $P = 0.008$; $z = 2.10$, $n = 9$; $P = 0.04$, respectively) in the Treatment Period in comparison with the Familiarity Period.

Feed pecking

There were no significant differences between or within treatment groups on any measure of feed pecking during the Familiarity or Treatment Period.

Affiliative pecking

On Day 1 of the Treatment Period a significant difference was seen between treatment groups in the amount of affiliative

pecking performed (Chi-square = 14.031, $df = 5$, $P = 0.015$): DC2 hens performed more affiliative pecking than C2 ($P = 0.05$) and DC1 hens ($P = 0.05$). There were no significant differences in the mean percentage of time spent in affiliative pecking between Familiarity and Treatment Periods within treatment groups.

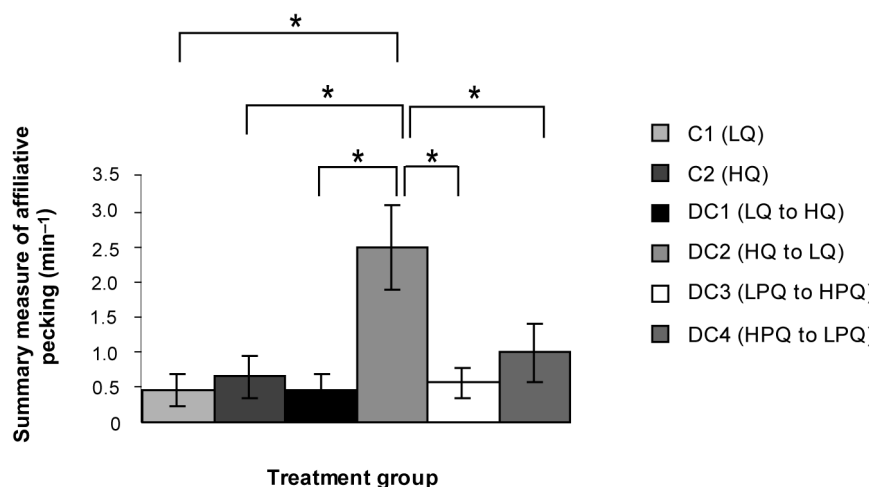
Total non-nutritional pecking

There were significant differences between groups during the Familiarity Period (Chi-square = 15.340, $df = 5$, $P = 0.009$; Figure 2) but not during Day 1 of the Treatment Period or the Treatment Period as a whole.

Within Treatment groups, birds from C2 did more non-nutritional pecking during the Treatment Period than in the Familiarity Period (Familiarity: $13.06 \pm 2.71\%$; Treatment: $28.95 \pm 3.22\%$; $t = -4.836$, $df = 8$, $P = 0.001$).

Figure 3

Affiliative pecking recorded by behaviour sampling over the duration of the Treatment Period.



Total inter-bird pecking and total injurious pecking

There were no significant differences between groups in the percentage of time spent in total inter-bird or total injurious pecking in either Familiarity or Treatment Periods.

Within Treatment groups, DC3 (Familiarity: $0.68 \pm 0.68\%$; Treatment: $1.41 \pm 0.72\%$; $z = -2.546$, $df = 8$, $P = 0.008$) and DC4 (Familiarity: $1.66 \pm 0.79\%$; Treatment: $4.39 \pm 1.71\%$; $z = -2.254$, $df = 8$, $P = 0.031$) groups both spent longer performing inter-bird pecks in the Treatment Period than in the Familiarity Period.

Beak inactivity

There were no significant differences between groups in the percentage of time spent in beak inactivity in either Familiarity or Treatment Periods. Within Treatment groups, birds from DC2 spent significantly less time in beak inactivity during the Treatment Period than during the Familiarity Period ($t = 2.459$, $df = 8$, $P = 0.039$).

Behavioural sampling

There were no significant differences for any of the analyses for the frequencies of particle pecking, mild feather pecking, severe feather pecking, vent pecking or furniture pecking. There were too few aggressive pecks for a meaningful statistical analysis.

Affiliative pecking

There were no significant differences between groups in affiliative pecking frequency during the Familiarity Period or on Day 1 of the Treatment Period. However, during the Treatment Period as a whole DC2 performed more affiliative pecking than all other groups (Chi-square = 11.965, $df = 5$, $P = 0.035$; Figure 3).

Within Treatment groups, DC2 birds performed more frequent affiliative pecking in the Treatment Period than they did in the Familiarity Period ($z = -2.666$, $n = 9$, $P = 0.004$).

Total injurious pecks

There were no significant differences between groups in the frequency of total injurious pecking in either Familiarity or Treatment Periods. Within Treatment groups, DC3 hens performed significantly more frequent injurious pecks during the Treatment Period than they did during the Familiarity Period ($z = -2.032$, $n = 9$, $P = 0.047$).

Total inter-bird pecks

There were no significant differences between groups in the frequency of total inter-bird pecking in either Familiarity or Treatment Periods. Within treatment groups, birds from DC4 performed more frequent inter-bird pecking during the Treatment Period than the Familiarity Period (Familiarity: $0.43 \pm 0.24 \text{ min}^{-1}$; Treatment: $1.92 \pm 1.02 \text{ min}^{-1}$; $z = -1.960$, $n = 9$, $P = 0.05$).

Discussion

Diet preference

The six pairs of Group P birds did not eat a significantly different amount of the two diets, HQ and LQ. Perhaps the birds were expressing a relative preference for a particular mixture of the diets, producing a nutrient intake somewhere between the two extremes available. Broiler chickens have been shown to have the 'nutritional wisdom' to choose to eat a mixture of two diets differing in protein content such that their protein requirements are met (Forbes & Shariatmadari 1994). In any case no 'absolute preference' (ie complete avoidance of one diet) for either diet was demonstrated.

Neophobia

Although dietary neophobia has been described in chickens (for example Murphy 1977; Jones & Roper 1997) and ducks (Desforges & Wood-Gush 1975), the phenomenon did not appear important in this experiment. Despite an abrupt and exaggerated commercial-style diet change, there were no

significant differences between treatment groups in the latency to peck at feed on Day 1 of the Treatment Period: in most pens, a bird pecked at the feed within a second of feed presentation. Also, there were no significant effects of diet change on feed consumption. This experiment supports the contention of Marples and Kelly (1999) that food neophobia in chickens is not very strong and can be easily overcome unless food is extremely unpalatable or toxic (Kare & Scott 1962; Forbes 1995). Dietary neophobia may therefore not be relevant in a commercial context. Experimentally, dietary neophobia is often reported when there is a dramatic change in the visual appearance of the feed (Murphy 1977; Jones 1986). This change in appearance is not found in the commercial context or in this experiment. Some previous experiments used naïve chicks, less accustomed to diet change, for example, Jones (1986). The commercially-reared chickens in this experiment had been phase fed four different diets prior to starting this experiment and hence were accustomed to dietary change. Such previous commercial experience of change may also reduce neophobia.

Absolute effects of diet

Hens eating a low-quality diet might behave differently to hens eating a high quality diet in which case it would be unsurprising if diet change resulted in behavioural change. Such a change could be said to result from the 'absolute' effects of the diet, as opposed to the 'relative effects' of feeding one diet sequentially after another. We did not find consistent behavioural differences between the two control groups of hens, C1 and C2, constantly fed diets LQ and HQ, respectively. Initially, during the Familiarity Period, birds from C1 performed more preening behaviour than birds from C2, and more non-nutritional pecking overall than birds from C2 but these effects did not persist into the Treatment Period.

Activity levels

An increase in activity may make adaptive sense in the natural environment of the chicken when food supplies become low, unpalatable or unsuitable: these behaviours increase the chance of discovering a new food source. There are several reports of increased activity in poultry in these types of circumstances (Hughes & Wood-Gush 1973; Nicol & Guilford 1991; Bruce Webster 1995; Zimmerman & Koene 1998; Haskell *et al* 2001; Hocking *et al* 2001). We found no effects of diet or diet change on overall moving, lying or perching but birds from DC 1 spent more time standing during the Familiarity Period when they were eating LQ feed than the Treatment Period when they were given HQ feed. We also found that beak activity increased in DC2 birds after diet change from HQ to LQ feed. Increases in beak activities such as foraging, cage and feather pecking have been observed in fasted layer hens (Bruce Webster 1995), restricted-fed broiler breeders (Hocking *et al* 2001, 2002), restricted-fed turkeys (Hocking *et al* 1999) and calcium-deprived pullets in battery cages (Hughes & Wood-Gush 1973). Dixon *et al* (2006) reported an increase in beak activities in layer chicks undergoing diet change to a less-preferred orange-flavoured feed from a more-preferred unflavoured feed. If particular sorts of diet

changes, such as diet changes to less-preferred feeds or less nutrient dense feeds (common practice in commercial phase feeding) increase the overall motivation to peck then this is one mechanism by which diet change might result in an increase in feather pecking. However, although DC2 birds engaged in more frequent beak activity after diet change, this was not specifically feather pecking.

Evidence of changes in pecking targets following diet change

Dixon *et al* (2006) reported an increase in pecking at box furniture, which coincided with a tendency to peck less frequently at food, in young chicks which had undergone a diet change from a preferred to a less-preferred diet. However, there was no evidence from either the scan samples or from behavioural samples of any increase in furniture pecking or litter pecking following diet change in this experiment. Perhaps young chicks have a greater propensity for these types of investigative pecking activities than older hens. Alternatively, such shifts in pecking target may occur only when birds are shifted to a significantly less-preferred diet, such as the orange-flavoured food used by Dixon *et al* (2006). In the current experiment no significant preferences for the two diets were recorded.

We found that hens from treatment group DC3 spent more time pecking other birds after diet change (there were significant increases in inter-bird pecking in scan samples and significant increases in total injurious pecking in behavioural samples, and trends towards increases in mild feather pecking from both types of samples). DC4 birds also showed a significant increase in inter-bird pecking after diet change. These findings are the first experimental support for the hypotheses that diet change may lead to an increase in feather-pecking behaviour. However, it is difficult to attribute these increases in feather pecking to a 'redirection' of feed pecks, since feed-pecking did not decrease after diet change in either group. It also remains unexplained why similar changes were not seen in DC1 and DC2 hens, which underwent similar diet changes but without oregano oil in the diets.

Affiliative pecking

There was strong evidence of an increase in affiliative pecking in DC2 hens following diet change. During the whole Treatment Period, DC2 hens that had undergone this diet change, performed more affiliative pecking than all other treatment groups, and there was also an increase in affiliative pecking within this group after diet change. DC4 hens underwent a similar behavioural change, but of a lower magnitude: it is possible that the use of 'masking' oregano oil minimised this behavioural effect of the diet change. These data are consistent with the hypothesis that diet change from a high to low nutrient density resulted in increased affiliative pecking. There are similarities between this finding, and the previously reported increase in affiliative pecking found in layer chicks on the first day following a diet change from an unflavoured feed to a less-preferred orange-flavoured feed (Dixon *et al* 2006).

Unfortunately, little is known about the motivation underlying affiliative pecking. Savory and Mann (1997) noted

that increases in preening and feather pecking were synchronous during development in a range of layer strains and it was speculated that that attention to the plumage of other birds through allopreening pecks may lead to later increases in feather pecking. Riedstra and Groothuis (2002) found that high feather-pecking strain chicks performed more 'social pecks' than low feather-pecking strain chicks. Social pecks were defined as 'non-aggressive pecks towards cagemates not directed towards plumage': it is likely that these consisted mostly of affiliative pecks. The authors also found that mild feather pecking occurred more frequently when chicks were resting and engaging in other forms of social pecking than when foraging or dustbathing. This is corroborated by the findings of a commercial study of 36 flocks (Zimmerman *et al* 2006) which showed a significant positive correlation between allopreening and feather pecking. Affiliative pecks are a component of 'allopreening' (Blokhuys & Arkes 1984; Leonard *et al* 1995; Savory & Mann 1997), which is thought to play a role in reducing aggression and the formation of social bonds (Harrison 1965; Wood-Gush & Rowland 1973). In a study of small groups of hens and cockerels by Leonard *et al* (1995), 77% of allopreens were directed at the head, bill or comb and wattle and would be classified as affiliative pecks in the present experiment. Allopreens were the second commonest form of allopecking after aggressive pecks, accounting for about 27% of all allopecks seen. Therefore over 20% of all inter-bird pecks observed in Leonard *et al*'s study were likely to be affiliative pecks. In our Treatment Period, 24% of all inter-bird pecks during scan sampling and 43% during behavioural sampling were affiliative.

Affiliative pecking frequently seemed to be directed at feed particles on the recipient's beak. It is possible that these pecks might be redirected foraging pecks towards a potential feed source. The increase in affiliative pecking was noted in birds which had undergone a change from HQ to LQ feed. The LQ feed had lower oil but slightly higher moisture content, which may have affected its tendency to stick on the beak. If anything, we would expect that the lower oil feed would be less sticky but we did not formally test this aspect of diet consistency and so cannot exclude the possibility that food particles were differentially available for pecking. Alternatively, these pecks might represent a form of sampling conspecifics' food intake, in an attempt to identify superior food sources. An investigation of these possibilities would be of great interest.

Animal welfare implications

Identifying risk factors for injurious pecking in laying hens is a high animal welfare priority. Diet change has previously been identified as a risk factor for feather pecking in laying hens. However, it is important to understand the mechanisms that may underlie such associations if sensible management changes are to be implemented. It has been suggested that neophobia to new diets might precipitate pecking, but we found no evidence of dietary neophobia in the present study. We also found no behavioural changes consistently attributable to the absolute protein levels of the diets. We did find an

increase in beak activity in birds which had undergone a diet change from a higher nutrient density to a lower nutrient density diet and a stronger effect showing that this diet change was associated with an increase in affiliative pecking. Masking the diet change using oregano oil as flavouring reduced the effects of the change on both rises in beak activity and affiliative pecking. The relationship between affiliative pecking and the subsequent development of injurious pecking requires longer-term investigation.

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