British Poultry Science (2001) **42:** 33–42

Development of pecking damage in layer pullets in relation to dietary protein source

D.E.F. McKEEGAN[1], C.J. SAVORY[2], M.G. MacLEOD AND M.A. MITCHELL

Roslin Institute (Edinburgh), Scotland

Abstract 1. In recent years, the UK egg industry has become increasingly dependent on plant protein sources, in particular soyabean meal, and it has been suggested that this trend (and/or the concomitant absence of animal protein in layer diets) might be causally related to increased feather pecking and cannibalism.
2. This study examined the development of pecking damage in relation to dietary protein source, by rearing 12 groups of 12 layer pullets to 24 weeks of age on diets based on 'animal' (fishmeal) or 'plant' (soyabean meal) protein.
3. Damaging pecking began at 6 weeks of age, in three groups (one plant and two animal). Injurious pecking began at 18 weeks of age, and affected four groups (two plant and two animal).
4. Greater numbers of vigorous pecks/pulls were observed in plant protein groups throughout the experiment, although they were significantly higher only between 13 to 16 weeks of age. Pecking damage scores did not differ between treatments.
5. Dietary protein source did not affect plasma oestradiol, progesterone or egg production.
6. These results do not support the notion that inclusion of fishmeal in laying hen diets prevents or alleviates feather pecking and cannibalism.

INTRODUCTION

Modern layer diets rely almost exclusively on plant protein sources, with widespread use of feedstuffs such as soyabean and rapeseed meal. Reasons for the move away from animal protein sources include the UK ban on the use of meat and bone meal (as part of the programme designed to eradicate bovine spongiform encephalopathy (BSE)) and the relatively high cost of fishmeal. In recent years, there has been increasing speculation that extensive use of plant-protein-based diets might be causally related to increased pecking damage in laying hens. In its 1997 Report on the Welfare of Laying Hens, the Farm Animal Welfare Council stated (without any conclusive evidence) that 'lack of animal protein in the diet predisposes the flock to injurious pecking leading to cannibalism and death' (Paragraph 41). They recommended 'further research work to identify and quantify the factors in animal protein responsible for reducing injurious behaviour in laying hens' (Paragraph 42).

In previous studies, low protein diets (regardless of source) have been associated with increased feather pecking and mortality attributable to cannibalism (Cain *et al.*, 1984; Ambrosen and Petersen, 1997) but protein source effects on the incidence of pecking damage have received little attention. Some animal/plant protein source comparisons have been carried out, examining effects on performance traits such as egg production (Mundheim and Opstvedt, 1981; Al Bustany and Elwinger, 1987*a*) and egg quality (Al Bustany and Elwinger, 1987*b*). Atteh and Ajakaiye (1993) offered laying hens one of three additional protein sources (soyabean meal, fishmeal or bloodmeal) in a 'cafeteria' type feeding system and, while cannibalism was observed in all groups, it was worst in the groups with access only to bloodmeal or soyabean meal. In the only study directly aimed to examine effects of dietary protein source on pecking damage, Savory *et al.* (1999) compared pecking damage in growing bantams (0 to 6 weeks of age) fed isonitrogenous diets based on either plant (soyabean meal), animal (bloodmeal, fishmeal and hydrolysed feather meal) or semipurified (casein) protein. No difference in pecking damage scores between treatments was observed. Thus, an effect of dietary protein source on the incidence of pecking damage in layers has never been demonstrated experimentally, although there have been anecdotal reports of outbreaks of feather pecking and cannibalism after changes in the diet from mainly animal to mainly plant protein (for example, Curtis and Marsh, 1992).

[1]Correspondence to: Dorothy E.F. McKeegan, Welfare Biology Group, Roslin Institute, Roslin, Midlothian, EH25 9PS. E-mail: dorothy.mckeegan@bbsrc.ac.uk
[2]National Centre for Poultry Studies, SAC Auchincruive, Ayr KA6 5HW, Scotland.
Accepted for publication 21st July 2000.

ISSN 0007–1668(print)/ISSN 1466–1799(online)/01/010033–10 © 2001 British Poultry Science Ltd
DOI: 10.1080/00071660020035055

It has been assumed that any suppressive effects on pecking induced by fishmeal are due to something beneficial found only in animal protein sources, such as the 'animal protein factor' vitamin B_{12} (Bolton and Blair, 1974). However, it is also conceivable there could be something detrimental in plant protein sources. Phytoestrogens, plant compounds which mimic steroidal oestrogens, are found in many plant species, some of which are used as feedstuffs for poultry (Sheehan, 1995; Knight and Eden, 1995). Soyabeans in particular are a rich source of the isoflavonoid phytoestrogens genistein and diadzein, which have oestrogenic potencies of 0·084% and 0·013% compared to oestradiol. Despite these relatively low potencies, phytoestrogens may be present in high concentrations in some feedstuffs (1800 mg/kg isoflavones in soyabeans, for example (Bingham *et al.*, 1998)) and could therefore be important biologically.

The biological effects of phytoestrogens appear to depend on both species and concentration (Shemesh and Shore, 1987). Although nothing is known of the behavioural effects of these compounds in poultry, there is evidence that several phytoestrogens are biologically active in birds, such as genistein and diadzein (Leopold *et al.*, 1976, wild quail), equol (Cayen *et al.*, 1965; Axelson *et al.*, 1984, domestic fowl), coumestrol (Mohsin and Pal, 1977, Beguin and Kincaid, 1984, domestic fowl) and zearalenone (Allen *et al.*, 1981; Shemesh and Shore, 1987, domestic fowl). Adverse effects of phytoestrogens on fowl reproductive development have been reported, with laying hens fed diets containing high levels of coumestrol (an isoflavone) exhibiting late sexual maturation, depressed egg production and low egg weight (Mohsin and Pal, 1977). Importantly, elevated plasma oestradiol concentrations have been reported in laying hens fed diets containing soyabean meal (compared to a diet containing fishmeal as the main protein source) (Maurice *et al.*, 1979; Akiba *et al.*, 1982). The increased plasma oestradiol seen in these studies suggest that phytoestrogens were acting antagonistically, increasing gonadotrophin secretion and gonadal activity in a similar way to the anti-oestrogen tamoxifen (Lazier, 1987; Jaccoby *et al.*, 1992). Phytoestrogens in soyabean meal have also been found to act agonistically in other species, for example, increasing vitellogenesis in cultured Siberian Sturgeon (Pelissero *et al.*, 1991).

Several authors have observed that pecking damage begins or increases at sexual maturation (Allen and Perry, 1975; Blokhuis, 1991; McKeegan and Savory, 1998; Bilcik and Keeling, 1999), when plasma concentrations of gonadal hormones are elevated. Such temporal associations provide circumstantial evidence for a relationship between hormonal state and the incidence of pecking damage but only one study has provided evidence for a causal link. Hughes (1973), reported increased pecking damage in juvenile pullets treated chronically with a combination of oestradiol and progesterone. In addition, a demonstrated association exists between oestradiol and amount of activity (Horne and Wood-Gush, 1970), which in turn is related to the incidence of damaging pecking (Keeling and Jensen, 1995; Savory and Griffiths, 1997; Savory and Mann, 1997). It is possible, therefore, that the presence of oestrogen-like compounds in plant protein sources could influence pecking behaviour. If this is the case, then effects of phytoestrogens could depend on the stage of sexual maturation and this may explain why no effects were observed in the juvenile bantams studied by Savory *et al.* (1999).

This study aimed to investigate experimentally whether dietary protein source has any effect on the development of feather pecking and cannibalism in commercial layer pullets, by rearing birds on isonitrogenous and isocaloric diets based on either plant protein (mainly soyabean meal) or animal protein (containing fishmeal). Because of previous unpredictability observed in pecking behaviour (McKeegan and Savory, 1998), 12 groups of birds were used to compare two dietary treatments (six groups per treatment) to increase the likelihood of obtaining meaningful results. Effects of dietary protein source on behaviour (particularly bird to bird pecking), growth and egg production were determined to 24 weeks of age. Plasma oestradiol and progesterone concentrations were also measured in a subset of birds between 10 and 24 weeks of age to assess any effects on hormonal state of inclusion of soyabean meal in the diet.

MATERIALS AND METHODS

Subjects and husbandry

One hundred and forty-four ISA Brown pullets were allocated at random on the day of hatch into 12 groups of 12 chicks and placed in 12 adjacent pens measuring 1·6 m×1·2 m. The pens were in a windowless house and each had solid walls, wood shavings litter, a red plastic bell-type automatic drinker and a metal food hopper. Food and water were provided *ad libitum*. Light was provided from eight tungsten ceiling lights containing 100 W bulbs and an additional 100 W light source present in each pen. Light intensity at floor level was between 84 and 144 lux (overall mean 116 lux). The light regimen was 10L: 14D until 14 weeks, after which the light period was increased by 1 h in each of the next 4 weeks until the photoperiod

Table 1. *Calculated values for crude protein (CP), energy (TME) and calcium in the animal and plant protein starter, grower and layer diets*

		CP (g/kg)	TME (MJ/kg)	Calcium (g/kg)
Starter (0–6 weeks)	Animal	178	11·8	9·5
	Plant	180	12·0	9·0
Grower (7–16 weeks)	Animal	149	12·0	8·1
	Plant	150	12·0	8·0
Layer (17–24 weeks)	Animal	161	11·8	32·6
	Plant	163	11·7	32·4

Table 2. *Complete formulations of the starter, grower and layer animal (A) and plant (P) protein diets*

	Starter		Grower		Layer	
Dietary constituent (g/kg)	A	P	A	P	A	P
Barley meal				100		25
Wheat meal	606	639	694	595	734	606
Wheat feed	246	75	200	106		
Soyabean meal		169		96		104
Rapeseed meal		50		40		50
Fish meal	94		68		80	
Grass meal					50	49·7
Maize oil	11·7	23·6	15	23	18	35
Maize gluten meal	21	1·5			34	30
Lysine	0·9		0·25		0·85	0·55
DL-Methionine						0·25
Tryptophan	0·4		0·15		0·15	
Limestone flour		1·85		0·4	64	71
Dicalcium phosphate		19·2	3·1	19·6		9
Sodium chloride	2	2·85	2·5	3	2	2·5
Vitamin/mineral mix	8	8	7	7	7	7
Pellet binder	10	10	10	10	10	10

was 14 h at 18 weeks. Ambient temperature in the house ranged from 15° to 24°C (mean 20°C), although additional heating was provided at chick level until 6 weeks of age.

At 1-d-old, groups were assigned at random to animal or plant protein treatments, six groups per treatment, and as such were fed starter (0 to 6 weeks), grower (7 to 16 weeks) and layer (17 to 24 weeks) pelleted diets based on either fishmeal (animal) or soyabean meal (plant). The animal and plant diets were equalised as far as possible for total crude protein, energy and calcium in the three diet types (Table 1); full formulations are shown in Table 2. The crude protein/metabolisable energy ratios for the animal and plant diets were 15·1 and 15·0 g/MJ for the starter diets, 12·5 and 12·5 g/MJ for the grower diets, and 13·7 and 13·9 g/MJ for the layer diets. Vitamins and minerals were equalised and special care was taken to equalise the amino acid tryptophan, which was shown to influence pecking behaviour previously (Savory *et al.*, 1999). All diets were produced by Roslin Nutrition Ltd, and all feedstuffs used were the same as those supplied to the poultry industry. The pure fishmeal used was 'Norvite' (66% protein, 8% oil). Soyabean meal (48% protein) and rapeseed meal were included in the plant protein diet, because rapeseed meal is increasingly included in commercial plant protein diets (Barbour and Sim, 1991; Lee *et al.*, 1995).

Measurements of growth and pecking damage

All birds were weighed in alternate weeks from 2 to 24 weeks and were inspected for pecking damage at the time of weighing. Areas of the body subject to pecking damage were the back, tail, wings, neck (including upper breast), head and comb. The plumage was examined and damage to each body area was scored on a scale from 0 to 5 as described by Savory *et al.* (1999). Any fresh wounds were sprayed with a (blue coloured) proprietary antibiotic aerosol (Engemycin, Mycofarm UK) which discouraged further pecking and reduced the risk of wound infection. All birds were inspected twice daily and any bird found with a wound exceeding 2 cm^2 in area was culled. For ethical reasons, it was decided that the level of mortality/culling due to injurious pecking at which a group of birds should be removed from an experiment should be 33% (four out of 12 birds). This work was carried out under the authority of a UK Home Office Licence.

Behaviour observations

Individual birds in each group were identified by unique combinations of coloured leg rings and, up to 3 weeks of age, by unique markings on the head made with permanent marker pen.

Observations to determine the behavioural time-budget were carried out in alternate weeks from 2 to 22 weeks of age. Each group was

observed once in each of four observation sessions (two-mornings and two afternoons), in random order. Data were recorded on a hand held computer (Atari Portfolio) using the KEY-BEHAVIOUR package (Deag, 1994). Observations of each group consisted of a single 'on the dot' recording (Martin and Bateson, 1986) of behaviour for every bird in each of 6 consecutive min (hence, six scans ×12 birds giving 72 observations per group per session). The (11) mutually exclusive activities recorded were sitting (only), standing (only), walking, pecking or scratching litter, feeding (or feeder directed), drinking (or drinker directed), preening while standing, preening while sitting, dustbathing, pecking another bird non-aggressively and pecking another bird aggressively.

Additional observations of bird-to-bird pecking were carried out weekly from 1 to 24 weeks of age. Pens were observed in random order, twice weekly (one morning and one afternoon session). Every bird in each pen was observed individually for 1 min, each pen being observed for a total of 12 min per session. During each 'focal' observation, every feather peck and feather pull given or received by the subject was recorded on a checksheet. Pecks were defined as either aggressive (forceful, aimed at the head or back of the recipient, rarely repeated, recipient immediately withdraws) or non-aggressive (gentle or vigorous, aimed at any part of the body, often repeated, recipient may eventually withdraw). Data from morning and afternoon sessions were combined to give total counts for each pen in each week.

Egg production

Egg production was monitored on a per-pen basis, with eggs collected daily from the litter floor and marked with the date and pen number. Eggs were counted and bulk weighed at the end of each week to provide pen means for egg number and egg weight.

Blood sampling and assays

Serial blood samples (2 ml) were obtained by venepuncture (wing vein) from two birds in each group at 2 week intervals from 12 to 24 weeks of age. Plasmas were prepared immediately by chilled centrifugation (5°C) at 1500 *g* for 10 min and stored at –20°C until analysis.

Plasma oestradiol was determined using a commerically available double antibody radioimmunoassay kit (Pantex ^{125}I Estradiol, Biogenesis Ltd, Poole, UK) with a modified extraction. This was necessary because laying hen plasma contains considerably higher concentrations of lipid and lipoproteins (up to 30 mg/ml) than human plasma. The extraction procedure was based on that described by Webb *et al.*, (1985); full details are described in McKeegan (2000). Mean extraction efficiency, as determined by recovery of tritiated oestradiol calculated in every tube was 73±0·06%. By this means, individual recoveries were taken into account in the final calculation of oestradiol concentration for each sample. Plasma progesterone concentration was determined using a commercially available coated-tube radioimmunoassay kit (Coat-a-count Progesterone, Diagnostic Products Corporation).

Statistical analyses

Comparisons were carried out by *t* test to determine treatment effects at each age on body weight, pecking damage score, egg production and plasma hormone concentrations. Data from behaviour observations were summarised into age ranges (weeks 2 to 4, weeks 6 to 10, weeks 12 to 16 and weeks 18 to 22 for the ethogram, and weeks 1 to 4, 5 to 8, 9 to 12, 13 to 16, 17 to 20 and 21 to 24 for pecking counts). To compare overall pen means *t* test were also carried out on counts of gentle and vigorous pecks across the whole experiment (1 to 24 weeks).

RESULTS

Bodyweight

Groups receiving the animal protein diet were significantly heavier than those fed the plant protein diet at every age from 4 weeks onwards (*t* tests with pen means, $P<0{\cdot}05$, $n=12$). At the end of the experiment at 24 weeks, birds fed the animal protein diet weighed 1920±51 g (group mean±SE) while those fed the plant protein diet weighed 1815±45 g. Within treatments, variation in bodyweight between birds was small (mean Coefficients of Variation across ages for animal and plant protein of 0·04 and 0·03, respectively).

Behaviour observations

Standing, walking, litter-directed behaviour and feeding took up the largest portions of the time budget in all age ranges. Dietary treatment effects on behaviour were observed in some activities at some ages (Figure 1). Animal-protein groups spent significantly more time standing than those receiving the plant-protein diet, between 0 to 4 weeks ($P<0{\cdot}01$, $n=12$) and 6 to 10 weeks ($P<0{\cdot}05$). Between 0 to 4 weeks, plant-protein groups spent more time in litter directed behaviour than did animal-protein groups ($P<0{\cdot}05$, $n=12$). Between 6 to 10 weeks, plant-protein birds spent more time pecking other birds (aggressively or non-aggressively) than did

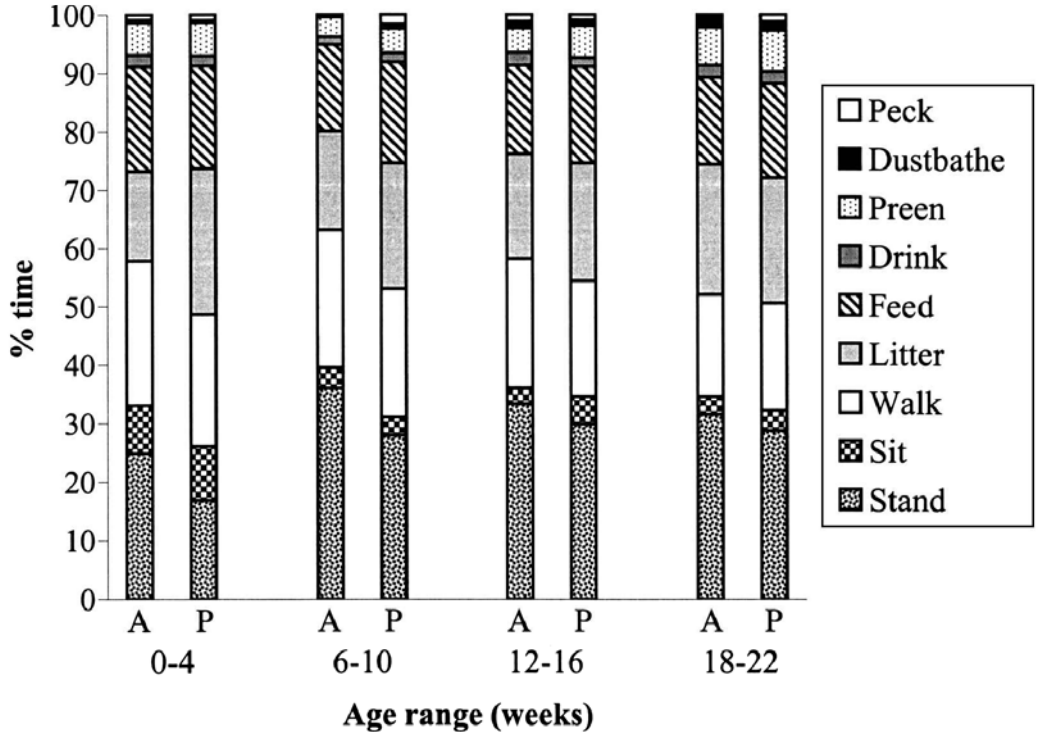

Figure 1. *Mean time spent in ethogram behaviours between 0 and 22 weeks in groups of pullets receiving diets based on animal (A) or plant (P) protein (6 groups per treatment).*

those on the animal protein diet ($P<0{\cdot}05$, $n=12$). No significant differences were observed between treatments at 12 to 16 weeks and 18 to 22 weeks.

Gentle pecks were the commonest bird-to-bird pecks observed, accounting for 74% of all pecks seen. There were no significant differences between treatments in numbers of gentle pecks given and received in any age range, and an increase was seen in both treatment groups at the onset of lay (17 to 20 weeks, Figure 2). Consistently higher numbers of vigorous pecks and pulls were seen in plant-protein groups throughout the experiment (Figure 3) but this difference was significant only in the 13 to 16 week age range ($P<0{\cdot}05$, *t*-test, $n=12$). Numbers of aggressive pecks increased after sexual maturity (data not shown) but were not affected by dietary treatment. Comparisons of pen means by *t* test for counts of gentle and vigorous pecks across the whole experiment (1 to 24 weeks) did not reveal any significant differences between groups reared on the animal or plant protein diet.

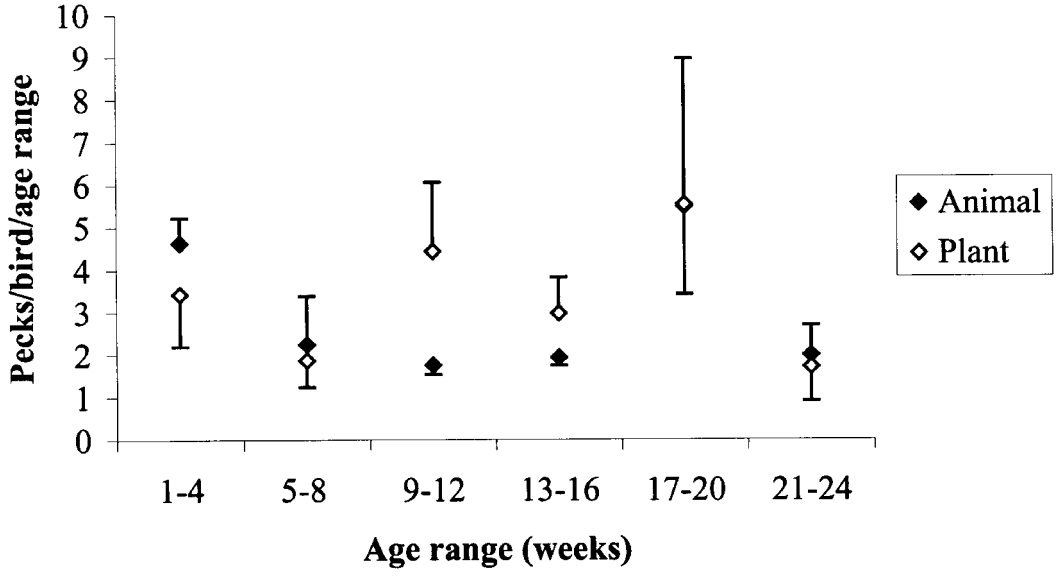

Figure 2. *Age related changes (across 6 age ranges) in mean numbers of observed gentle pecks (± SE) in groups of pullets receiving diets based on either animal or plant protein (6 groups per treatment).*

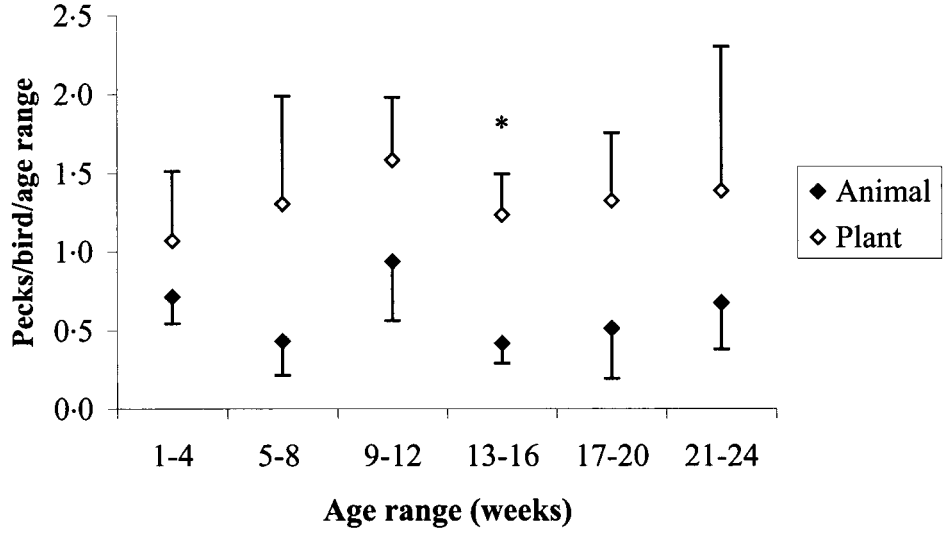

Figure 3. *Age related changes (across 6 age ranges) in mean numbers of observed vigorous pecks/pulls (± SE) in groups of pullets receiving diets based on either animal or plant protein (6 groups per treatment). *P<0·05.*

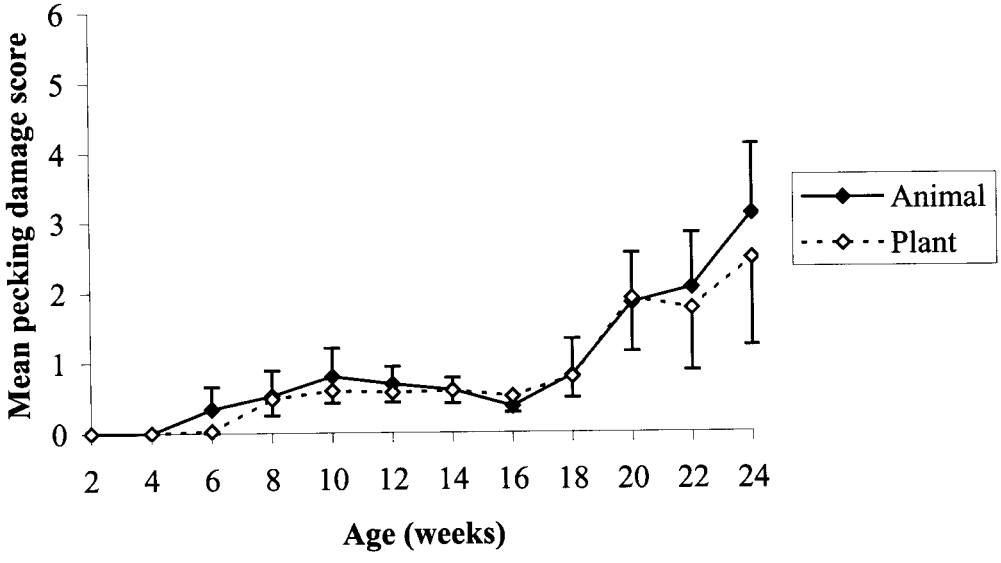

Figure 4. *Mean pecking damage scores (± SE) between 2 and 24 weeks of age in in groups of pullets receiving diets based on either animal or plant protein (6 groups per treatment).*

Pecking damage scores

Pecking damage scores did not differ significantly between treatments at any age, and did not reflect the increased vigorous pecking/pulling observed in plant protein groups. Pecking damage was first seen at 6 weeks of age (Figure 4), when scores were recorded in three groups, Pen 4 (plant), Pen 7 (animal) and Pen 10 (animal). Pecking in juveniles was targeted at the lower back, tail and wings, and was not severe. Damage scores were low until 16 weeks of age and increased consistently thereafter. Cannibalism first appeared at 18 weeks of age and 10 deaths (including seven culls) occurred between 18 and 24 weeks. While pecking damage scores were recorded in all groups, injurious pecking took place in only Pens 2 (plant), 5 (plant), 7 (animal) and 12 (animal). Pens 2, 5 and 7 were removed from the experiment prematurely to prevent further mortality (see Methods). Vent pecking was observed in both treatment groups and accounted for seven of the 10 deaths/culls attributable to cannibalism (five animal, two plant). Other cannibalistic attacks were targeted at the tail.

Egg production

Egg production commenced in some groups at 16 weeks of age and all groups were in lay by 17 weeks. Egg production rose steadily, approaching 0·8 eggs hen/d by the end of the experiment at 24 weeks, and neither it nor mean egg weight was affected by dietary treatment at any age.

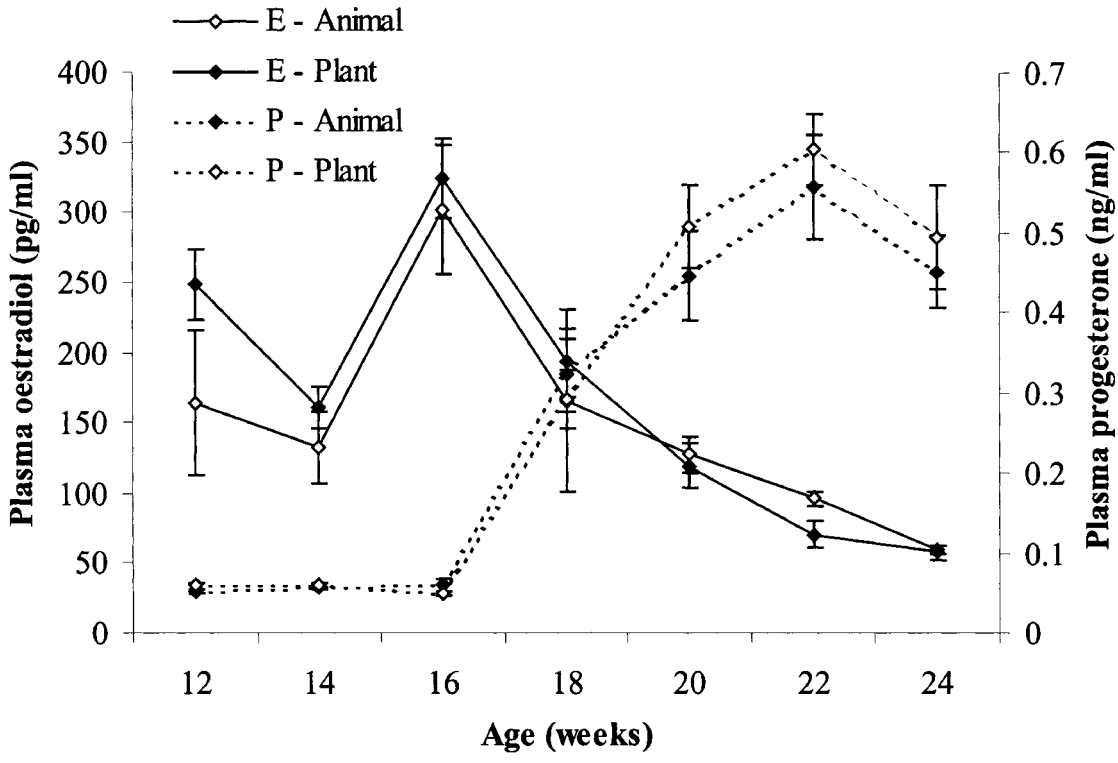

Figure 5. *Mean plasma oestradiol (E) and progesterone (P) concentrations (± SE) between 12 and 24 weeks of age in pullets receiving diets based on animal or plant protein (n=12 per treatment at each age).*

Plasma hormones

Plasma oestradiol concentration was not affected by dietary treatment and rose from 100 to 200 pg/ml at 14 weeks of age to around 300 pg/ml at 16 weeks of age, and then fell again, in both treatment groups (Figure 5). Plasma progesterone was also unaffected by dietary protein source and increased rapidly between 16 and 20 weeks of age (Figure 5) from less than 0·1 ng/ml to a peak of 0·6 ng/ml.

DISCUSSION

Consistently heavier bodyweights were observed in pullets receiving the animal protein diet. Although attempts were made to measure food intake early in the experiment, this was abandoned as substantial food spillage in several pens made accurate measurement impossible. Some authors have reported improved growth in layers fed diets containing fishmeal (Mundheim and Opstvedt, 1981), while others found no beneficial effect (Cantor and Johnson, 1983; Al Bustany and Elwinger, 1987*a*). Because fishmeal is known to be very palatable and preferences for it have been demonstrated (Alenier and Combs, 1981; Cantor and Johnson, 1983; Atteh and Ajakaiye, 1993), increased food intake seems the most likely explanation for the higher bodyweights in animal protein groups. Overall, the body weight differences observed were small and tended to diminish with age, and were not accompanied by differences in age at onset of lay or egg production.

Differences between the animal and plant protein groups in time spent in ethogram behaviours were apparent between 0 to 4 weeks (standing and litter directed behaviour) and 6 to 10 weeks (standing and bird to bird pecking). Less time spent standing (doing nothing else) and more time spent pecking and scratching litter could indicate greater activity in plant protein groups, although no difference was observed between treatments in time spent sitting (which is often used as a measure of inactivity). Increased litter-directed activity in juveniles has been associated with reduced feather pecking later in life (Blokhuis and van der Haar, 1989; Blokhuis and Beuving, 1993; Blokhuis and Wiepkema, 1998). However, there was some evidence (based on counts of vigorous pecks/pulls) that damaging pecking was greater in plant-protein groups at the onset of lay, and time spent in bird to bird pecking (aggressive and non-aggressive) was significantly higher in plant protein groups between 6 to 10 weeks of age.

Detailed observations of pecking behaviour revealed no treatment effects on numbers of gentle, non-damaging feather pecks, and an increase in gentle pecks was seen in both treatments at the onset of lay (17 to 20 weeks). This age-related increase probably reflects an increase in dustbathing activity (during which

dustbathing individuals are the recipients of large numbers of gentle pecks aimed at particles on the plumage surface). By contrast, consistently higher numbers of vigorous, potentially damaging pecks and pulls were observed in plant-protein groups throughout the experiment (significant at 13 to 16 weeks). The results of pecking observations, therefore, tend to support the notion that damaging pecking is promoted by plant-protein-based diets. Atteh and Ajakaiye (1993) also reported more pecking damage in birds with access to a soyabean protein supplement compared to those with access to fishmeal.

Pecking damage was first seen at 6 weeks, remained low to 16 weeks (onset of lay) and increased consistently thereafter. Mortality attributable to cannibalism (including vent pecking) occurred in both animal and plant protein groups. No differences were observed between treatments in pecking damage scores, which did not reflect the increased vigorous pecking/pulling observed in plant-protein groups. It may be that not all vigorous pecks/pulls actually result in damage.

Egg production was unaffected by dietary protein source in this experiment, both in terms of egg number and egg weight. Previous studies comparing soyabean meal and fishmeal-based diets have reported no effects on egg production (Akiba *et al.*, 1982; A1 Bustany and Elwinger 1987a), although Mundheim and Opstvedt (1981) found improved egg production in layers fed a diet containing fishmeal.

There was no evidence of elevated plasma oestradiol or progesterone with the plant-protein diet. These results are in contrast to previous reports of increased plasma oestrogen, progesterone and lipid in laying hens fed maize–soyabean diets. Maurice *et al.* (1979) found significantly higher plasma oestrogen and lipid in hens fed a diet containing 12% soyabean meal compared to those fed a maize fishmeal diet. Akiba *et al.*, (1982) also reported increased plasma oestradiol when 18% soyabean meal was included in the diet. The starter, grower and layer diets used in the current experiment contained 16·9%, 9·6%, and 10·4% soyabean meal respectively, and the lower soyabean inclusion in the grower and layer diets may account for the lack of oestradiol/progesterone response. Concentrations of phytoestrogens present in plant material are known to be variable and dependent on plant age, growth rate, geographical location and storage conditions after harvesting (Leopold *et al.*, 1976). It is possible that one or all of these factors influenced the concentrations of phytoestrogens present in the soyabean meals used in this and previous dietary protein source comparisons. In the absence of direct measurement of phytoestrogens, it is impossible to say whether wide variation in these compounds exists in soyabean meal used for poultry diets. Presumably, the inclusion of rapeseed meal (which does not contain phytoestrogens) in the plant protein diets had a diluting effect.

In discussing the oestrogenic potential of the diets used in this experiment, it is important to consider that fishmeal has been identified as a dietary source of steroid hormones. Pelissero *et al.* (1989) discovered relatively high concentrations of oestradiol, oestrone and androgens in Norwegian fishmeal (similar to that used in this experiment) and noted wide variation between batches in steroid content. Although steroids in fishmeal are present at lower concentrations than phytoestrogens in soyabean meal (ng/100 g compared to mg/100 g), their greater potency may make them as important biologically. Hence, the presence of oestrogenic compounds in both diets could have obscured behavioural or physiological differences. However, observed plasma concentrations of oestradiol and progesterone were within the ranges expected for age (Senior, 1974; Williams and Sharp, 1977).

Although every attempt was made to ensure that the diets used in this study were comparable to commercial feeds, strictly speaking this experiment provides information limited to the exact formulations used. Nevertheless, this study represents the first attempt to examine the effects of dietary protein source on pullet behavioural development. Some anecdotal reports of an effect of dietary protein source on pecking damage describe a change in the diet, for example from mainly animal to mainly plant protein (Curtis and Marsh, 1992). This was not examined in the present study, where pullets were fed animal or plant-protein based diets from 1-d old to the onset of lay, an approach which allowed comparison of dietary effects on behaviour at all stages of development. A study designed to examine pecking behaviour after a change in dietary protein source would also be useful, providing direct comparison of diets in the same birds. However, care would need to be taken in the interpretation of results from such a study, because of known associations between rearing conditions and bird-to-bird pecking in adulthood (Blokhuis, 1991; Blokhuis and Van der Haar, 1992; Huber-Eicher and Wechsler, 1997) and the generally unpredictable occurrence of pecking damage in commercial laying strains.

In conclusion, these results do not provide convincing evidence to support the notion that the presence of fishmeal in layer diets suppresses pecking damage. While higher numbers of vigorous, potentially damaging pecks were seen in groups receiving the plant protein diet,

damaging pecking occurred in both animal and plant protein groups. It remains unclear whether the observed effects on pecking behaviour were due to absence of something beneficial in animal protein or the presence of a detrimental substance in plant protein. The paucity of significant results in this study may well be a consequence of examining an unpredictable problem with a relatively small number of birds. When applied on a commercial scale, however, the trends observed could account for the perceived worsening of pecking problems in layer flocks with increased use of plant-protein-based diets.

ACKNOWLEDGEMENTS

This work was supported by the British Egg Marketing Board Research and Education Trust.

REFERENCES

Akiba, Y., Jensen, L.S., Barb, C.R., & Kraeling, R.R. (1982) Plasma estradiol, thyroid-hormones and liver lipid-content in laying hens fed different isocaloric diets. *Journal of Nutrition*, **112:** 299–308.

Al Bustany, Z. & Elwinger, K. (1987*a*) Comparison between barley/fish meal- and maize/soybean meal-based diets with various lysine and protein levels fed to different strains of laying hens. *Acta Agriculturae Scandinavica*, **37:** 41–49.

Al Bustany, Z. & Elwinger, K. (1987*b*) The effects of diets based on barley and fish meal or maize and soybean meal on shell and interior quality and chemical composition of eggs from hens of different strains. *Swedish Journal of Agricultural Research*, **17:** 141–147.

Alenier, J.C. & Combs, G.F. (1981) Effects on feed palatability of ingredients believed to contain unidentified growth factors for poultry. *Poultry Science*, **60:** 215–224.

Allen, J. & Perry, G.C. (1975) Feather pecking and cannibalism in a caged layer flock. *British Poultry Science*, **16:** 441–451.

Allen, N.K., Mirocha, C.J., Aakhusallen, S., Bitgood, J.J., Weaver, G., & Bates, F. (1981) Effect of dietary zearalenone on reproduction of chickens. *Poultry Science*, **60:** 1165–1174.

Ambrosen, T. & Petersen, V.E. (1997) The influence of protein level in the diet on cannibalism and quality of plumage of layers. *Poultry Science*, **76:** 559–563.

Atteh, J.O. & Ajakaiye, A. (1993) Preference of laying hens for different protein sources in a cafeteria type feeding system. *Nigerian Journal of Animal Production*, **20:** 128–136.

Axelson, M., Sjovall, J., Gustafsson, B.E., & Setchell, K.D.R. (1984) Soya—a dietary source of the non-steroidal oestrogen equol in man and animals. *Journal of Endocrinology*, **102:** 49–56.

Barbour, G.W. & Sim, J.S. (1991) True metabolisable energy and true amino-acid availability in canola and flax products for poultry. *Poultry Science*, **70:** 2154–2160.

Beguin, D.P. & Kincaid, R.L. (1984) 3-Hydroxy-3-methyl-glutaryl coenzyme-a reductase-activity in chicks fed coumestrol, a phytoestrogen. *Poultry Science*, **63:** 686–690.

Bilcik, B. & Keeling, L.J. (1999) Changes in feather condition in relation to feather pecking and aggressive behaviour in laying hens. *British Poultry Science*, **40:** 444–451.

Bingham, S.A., Atkinson, C., Liggins, J., Bluck, L., & Coward, A. (1998) Phyto-oestrogens: Where are we now? *British Journal of Nutrition*, **79:** 393–406.

Blokhuis, H.J. (1991) Effects of rearing conditions on feather pecking in laying hens. *Applied Animal Behaviour Science*, **30:** 186–186.

Blokhuis, H.J. & Van Der Haar, J.W. (1989) Effects of floor type during rearing and of beak trimming on ground pecking and feather pecking in laying hens. *Applied Animal Behaviour Science*, **22:** 359–369.

Blokhuis, H.J. & Van Der Haar, J.W. (1992) Effects of pecking incentives during rearing on feather pecking of laying hens. *British Poultry Science*, **33:** 17–24.

Blokhuis, H.J. & Beuving, G. (1993) Feather pecking and other characteristics in two lines of laying hens. *Proceedings of the 4th European Symposium on Poultry Welfare*, pp. 145–157.

Blokhuis, H.J. & Wiepkema, P.R. (1998) Studies of feather pecking in poultry. *Veterinary Quarterly*, **20:** 6–9.

Bolton, W. & Blair, R. (1974) *Poultry Nutrition*. MAFF Bulletin 174 (HMSO, London).

Cain, J.R., Weber, J.M., Lockamy, T.A. & Creger, C.R. (1984) Grower diets and bird density effects on growth and cannibalism in Ring-Necked Pheasants. *Poultry Science*, **63:** 450–457.

Cantor, A.H. & Johnson, T.H. (1983) Effects of unidentified growth-factor sources on feed preference of chicks. *Poultry Science*, **62:** 1281–1286.

Cayen, M.N., Tang, G., & Common, R.H. (1965) Urinary conversion products of biochanin A and formononetin in the fowl. *Biochemica Et Biophysica Acta*, **111:** 349–357.

Curtis, P.E. & Marsh, N.W.A. (1992) Cannibalism in laying hens. *Veterinary Record*, **131:** 424.

Deag, J.M. (1994) KEYBEHAVIOUR, a program for recording behavioural data on the DIP Pocket PC and Atari Portfolio computer. Version TPPF3·4b.

Farm Animal Welfare Council (1997) *Report on the Welfare of Laying Hens*. Ministry of Agriculture, Fisheries and Food (HMSO, London).

Horne, A.R. & Wood-Gush, D.G.M. (1970) Exploratory activity following oestrogen treatment in ovariectomised brown leghorn females. *Revue de Comportement Animales*, **4:** 46–50.

Huber-Eicher, B. & Wechsler, B. (1997) Feather pecking in domestic chicks: its relation to dustbathing and foraging. *Animal Behaviour*, **54:** 757–768.

Hughes, B.O. (1973) The effect of implanted gonadal hormones on feather pecking and cannibalism in pullets. *British Poultry Science*, **14:** 341–348.

Jaccoby, S., Snapir, N., Rozenboim, I., Arnon, R., Meidan, R., & Robinzon, B. (1992) Tamoxifen advances puberty in the White Leghorn hen. *British Poultry Science*, **33:** 101–111.

Keeling, L.J. & Jensen, P. (1995) Do feather pecking and cannibalistic hens have different personalities? *Applied Animal Behaviour Science*, **44:** 257.

Knight, D.C. & Eden, J.A. (1995) Phytoestrogens—a short review. *Maturitas*, **22:** 167–175.

Lazier, C.B. (1987) Interactions of tamoxifen in the chicken. *Journal of Steroid Biochemistry*, **27:** 877–882.

Lee, K.H., QI, G.H. & Sim, J.S. (1995) Metabolizable energy and amino acid availability of flax and canola. *Poultry Science*, **74:** 1341–1348.

Leopold, A.S., Erwin, M., Oh, J., & Browning, B. (1976) Phytoestrogens: adverse effects on reproduction in California quail. *Science*, **191:** 98–100.

Martin, P. & Bateson, P. (1986) *Measuring Behaviour: an Introductory Guide*. (Cambridge University Press, Cambridge).

Maurice, D.V., Jensen, L.S., & Tojo, H. (1979) Comparison of fish meal and soybean meal in the prevention of fatty liver-hemorrhagic syndrome in caged layers. *Poultry Science*, **58:** 864–870.

McKeegan, D.E.F. (2000) An investigation of factors underlying the development of feather pecking and cannibalism

in commercial layer pullets. 178 pp. *PhD Thesis*, University of Edinburgh.

MCKEEGAN, D.E.F. & SAVORY, C.J. (1998) Behavioural and hormonal changes associated with sexual maturity in layer pullets. *British Poultry Science*, **39:** S6–S7.

MOHSIN, M. & PAL, A.K. (1977) Coumestrol in Lucerne (*Medicago sativa* L.) as a factor in egg production and its quality in White Leghorn pullets. *Indian Journal of Experimental Biology*, **15:** 76–77.

MUNDHEIM, H. & OPSTVEDT, J. (1981) The value of herring-type fish-meal and soybean meal as protein supplements to poultry diets based on different types of cereals. *Acta Agriculturae Scandinavica*, **31:** 287–298.

PELISSERO, C., CUISSET, B., & LE MENN, F. (1989) The influence of sex steroid in commercial fish meals and fish diets on plasma concentration of oestrogens and vitellogenin in cultured Siberian sturgeon *Acipenser baeri, Aquatic Living Resource*, **2:** 161–168.

PELISSERO, C., LE MENN, F., & KAUSHICK, S. (1991) Estrogenic effect of dietary soya bean meal on vitellogenesis in cultured Siberian Sturgeon *Acipenser baeri. General and Comparative Endocrinology*, **823:** 447–457.

SAVORY, C.J. & MANN, J.S. (1997) Behavioural development in groups of pen-housed pullets in relation to genetic strain, age and food form. *British Poultry Science*, **38:** 38–47.

SAVORY, C.J. & GRIFFITHS, J.D. (1997) Individual variation in rates of giving and receiving feather pecks in bantams and some behavioural correlates. *5th European Symposium on Poultry Welfare*, pp. 109–110.

SAVORY, C.J., MANN, J.S., & MACLEOD, M.G. (1999) Incidence of pecking damage in growing bantams in relation to food form, group size, stocking density, dietary tryptophan concentration and dietary protein source. *British Poultry Science*, **40:** 579–584.

SENIOR, B.E. (1974) Oestradiol concentration in the peripheral plasma of the domestic hen from 7 weeks of age until the time of sexual maturity. *Journal of Reproduction and Fertility*, **41:** 107–112.

SHEEHAN, D.M. (1995) The case for expanded phytoestrogen research. Presentation from the Second International Conference on Phytoestrogens. *Proceedings of the Society for Experimental Biology and Medicine*, **208:** 3–4.

SHEMESH, M., SHORE, & L.S. (1987) Non-steroidal oestrogens of dietary origin: activity, distribution and mechanism of action. *Israeli Journal of Veterinary Medicine*, **43:** 192–197.

WEBB, R., BAXTER, G., NORDBLOM, G.D., MCBRIDE, D., & SHAW, M.P.K. (1985) The measurement of testosterone and oestradiol-17 β using iodinated tracers and incorporating an affinity chromatography extraction procedure. *Journal of Steroid Biochemistry*, **23:** 1043–1051.

WILLIAMS, J.B. & SHARP, P.J. (1977) A comparison of plasma progesterone and luteinizing hormone in growing hens from 8 weeks of age to sexual maturity. *Journal of Endocrinology*, **75:** 447–448.