

An investigation of factors underlying the development of
feather pecking and cannibalism in
commercial layer pullets

Dorothy E. F. McKeegan

Doctor of Philosophy
University of Edinburgh
1999

For
Colin James Hamilton

Declaration

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

Dorothy E. F. McKeegan
November 1999

Acknowledgements

I am extremely grateful to my supervisors, Dr John Savory, Dr Malcolm Mitchell and Professor John Russell for their advice and encouragement during this project. I am also very grateful to the staff of Hut 11; Ailsa Carlisle, Richard Hunter, Dale Sandercock and Nick Gonet, for their practical help and for making my time in Hut 11 so enjoyable. I am particularly grateful to Colette Clarke for her friendship and support.

Other members of staff at Roslin Institute assisted me, and I am very grateful to Gerry Baxter and Heather McCormack for invaluable advice with assays, and to Dr Ian Dunn and John Bracken at the Large Animal Unit for help with hormone administration techniques. Thanks are due to Dr Murdo MacLeod who kindly formulated the animal and plant protein diets, Caroline McCorquodale who provided statistical advice and Dr Barry Hughes for useful comments on an earlier draft of the introduction to this thesis. Many thanks also to the Roslin farm staff and to Roddy and Norrie in the photography and graphics department for help throughout my project.

I am very grateful to the British Egg Marketing Board Research and Education Trust for funding this research, and for their enthusiastic support over the last three years.

Finally, a special thanks to my family and friends, particularly my parents and Andrew, for their constant support and encouragement during my studies.

Abstract

Feather pecking and cannibalism in laying hens continues to be a serious welfare and economic problem in the egg industry, and presents a major obstacle to the adoption of non-cage production systems. This project examined internal and environmental factors involved in the development of feather pecking and cannibalism in one commercial laying strain (ISA Brown), with particular attention to changes associated with sexual maturation at about 16 weeks of age.

A detailed study of behavioural and hormonal development in pen-housed pullets under constant environmental conditions revealed age-related changes in the number, type and targeting of bird-to-bird pecks. Feather pecking, which began in juvenile birds between 5 and 10 weeks of age, was associated (on a per pen basis) with more severe pecking damage after the onset of lay. This resulted from increased vigorous feather pecking/pulling, vent pecking and aggressive pecking. Times spent preening and dustbathing also increased at sexual maturity. Increased pecking damage at the onset of lay coincided with physiological changes, most closely with increased plasma progesterone concentration. Large variability between pens in the extent of pecking damage illustrated the unpredictability of pecking problems.

Investigation of the circumstantial link between damaging pecking and hormonal state (through experimental manipulations of the latter) proved to be problematic, and a direct causal link could not be demonstrated. The only evidence supporting such a link was from an experiment where acute administration of the anti-oestrogen tamoxifen resulted in reduced vigorous pecking and pulling (but not gentle pecking) at a novel pecking device (a bunch of string). There was no evidence of a relationship between birds' individual plasma hormone levels at 25 weeks of age and their feather pecking behaviour before or after sexual maturity.

Effects of dietary protein source on the development of damaging pecking were investigated, prompted by reports of increased pecking damage in layer flocks as a consequence of the widespread use of plant protein-based diets. In a comparison of pullets fed animal (fishmeal) or plant (soyabean meal) protein-based diets, there was some evidence (higher numbers of vigorous pecks/pulls) that damaging pecking was greater with the plant protein diet. There was no evidence of an oestrogenic effect of the plant protein diet (soya beans are a rich source of phytoestrogens), as indicated by plasma indicators of reproductive state or egg production.

Feather eating was common in both juvenile and adult layer pullets, with short feathers (<10cm in length) preferred. In juveniles, levels of feather eating (as indicated by depletion of moulted feathers on pen floors and the presence of feather material in droppings) were associated with pecking damage at 14 weeks on a per pen basis and reliably predicted the incidence of more severe pecking damage at the onset of lay. When birds from one experiment (in pens) were housed in cages and presented with short feathers, those previously identified as feather peckers ingested more feathers than non-peckers. In another, two-choice test, both peckers and non-peckers pecked at and ingested more unwashed feathers than washed ones, suggesting that the presence of preen oil may be a factor affecting attractiveness of feathers.

These findings identify external factors (feather eating and dietary protein source) which influence the aetiology of damaging pecking which may have important strategic implications for the egg industry.

Publications

- McKeegan, D.E.F. & Savory, C.J., 1997. Feather eating in commercial layer pullets and its putative role in the aetiology of pecking damage. In: Improving animal welfare through understanding behaviour. ISAE UK/EIRE Winter Meeting, 3rd December 1997, London.
- McKeegan, D.E.F. & Savory, C.J., 1998. Behavioural and hormonal changes associated with sexual maturity in layer pullets. *British Poultry Science*, 39 (Supplement): S6-S7.
- McKeegan, D.E.F. & Savory, C.J., 1999. Feather eating in layer pullets and its possible role in the aetiology of feather pecking damage. *Applied Animal Behaviour Science*, 65: 73-85.
- McKeegan, D.E.F. & Savory, C.J., 1999. Feather eating: a precursor of feather pecking? *Poultry Science*, 78 (Supplement 1), 97.
- McKeegan, D.E.F. & Savory, C.J., 1999. Behavioural and hormonal changes associated with sexual maturity in layer pullets. *Poultry Science*, 78 (Supplement 1), 142.
- McKeegan, D.E.F., Savory, C.J., Macleod, M.G. & Mitchell, M.A., 1999. An effect of dietary protein source on the development of pecking damage in layer pullets? *Poultry Science*, 78 (Supplement 1), 43.
- McKeegan, D.E.F., Savory, C.J., Macleod, M.G. & Mitchell, M.A., 1999. Is development of pecking damage in layer pullets influenced by dietary protein source? *British Poultry Science*, 40 (Supplement), S14-S15.
- McKeegan, D.E.F., Savory, C.J., Macleod, M.G. & Mitchell, M.A., (2000) Development of pecking damage in layer pullets in relation to dietary protein source. *British Poultry Science*, in press.

Contents

Declaration	i	
Acknowledgements	ii	
Abstract	iii	
Publications	iv	
Chapter 1	Introduction	1
1.1	Types of bird to bird pecking	2
	1.1.1 <i>Aggressive pecking</i>	2
	1.1.2 <i>Feather pecking</i>	3
	1.1.3 <i>Feather pulling</i>	3
	1.1.4 <i>Tissue pecking (cannibalism)</i>	3
	1.1.5 <i>Vent pecking</i>	4
1.2	Factors influencing pecking damage	4
	External factors	
	1.2.1 <i>Light</i>	5
	1.2.2 <i>Floor substrate</i>	6
	1.2.3 <i>Stocking density/group size</i>	8
	1.2.4 <i>Diet</i>	9
	1.2.5 <i>Environmental enrichment</i>	11
	1.2.6 <i>Production system</i>	12
	Internal factors	
	1.2.7 <i>Genetic variation</i>	13
	1.2.8 <i>Age/ development</i>	15
	1.2.9 <i>Hormonal state</i>	17
	1.2.10 <i>Fear</i>	19
1.3	Sexual maturation in laying hens: hormonal and behavioural changes	20
1.4	Thesis outline	21
Chapter 2	General materials and methods	23
2.1	Subjects	23
2.2	Husbandry	23
	2.2.1 <i>Housing and rearing</i>	23
	2.2.2 <i>Bird identification</i>	24
2.3	Growth measurements	25
	2.3.1 <i>Bodyweight</i>	25
	2.3.2 <i>Comb and wattle growth</i>	25
2.4	Behaviour	25
	2.4.1 <i>Ethogram</i>	26
	2.4.2 <i>Bird to bird pecking</i>	26
2.5	Pecking damage scores	27
2.6	Egg production	27
2.7	Blood samples	28

Chapter 2 (continued)

2.8	Assays	28
	2.8.1 <i>Triglyceride</i>	29
	2.8.2 <i>Zinc</i>	29
	2.8.3 <i>Gonadal steroids</i>	30
2.9	Hormone implants	30
	2.9.1 <i>Slow release pellets</i>	30
	2.9.2 <i>Silastic implants</i>	31
2.10	Surgical implantation	31
	2.10.1 <i>Local anaesthetic</i>	31
	2.10.2 <i>General anaesthetic</i>	33
2.11	Statistical analysis	33

Chapter 3 Behavioural and hormonal development of pullets **35**

3.1	Introduction	35
3.2	Methods	36
3.3	Results	37
	3.3.1 <i>Body weight</i>	37
	3.3.2 <i>Comb and wattle growth</i>	39
	3.3.3 <i>Ethogram</i>	39
	3.3.4 <i>Bird to bird pecking</i>	39
	3.3.5 <i>Pecking damage scores</i>	46
	3.3.6 <i>Egg production</i>	50
	3.3.7 <i>Plasma assays</i>	50
	3.3.8 <i>Comparisons between 'high' and 'low' pecking pens</i>	52
3.4	Discussion	53

Chapter 4 Linking behaviour and hormonal state **57**

4.1	Introduction	57
4.2	Experiment 1a – Combination pellet implants	59
	4.2.1 <i>Introduction</i>	59
	4.2.2 <i>Methods</i>	60
	4.2.3 <i>Results</i>	60
	4.2.4 <i>Conclusions</i>	65
4.3	Experiment 1b – Pellet investigation	66
	4.3.1 <i>Introduction</i>	66
	4.3.2 <i>Methods</i>	66
	4.3.3 <i>Results</i>	67
	4.3.4 <i>Conclusions</i>	67
4.4	Experiment 2a – First Silastic implant study	69
	4.4.1 <i>Introduction</i>	69
	4.4.2 <i>Methods</i>	69
	4.4.3 <i>Results</i>	70
	4.4.4 <i>Conclusions</i>	73

Chapter 4 (continued)

4.5	Experiment 2b – Second Silastic implant study	75
	4.5.1 <i>Introduction</i>	75
	4.5.2 <i>Methods</i>	75
	4.5.3 <i>Results</i>	76
	4.5.4 <i>Conclusions</i>	76
4.6	Experiment 3 – Oral administration	78
	4.6.1 <i>Introduction</i>	78
	4.6.2 <i>Methods</i>	79
	4.6.3 <i>Results</i>	79
	4.6.4 <i>Conclusions</i>	81
4.7	Experiment 4 – Effects of acute tamoxifen administration on novel object pecking	81
	4.7.1 <i>Introduction</i>	81
	4.7.2 <i>Methods</i>	82
	4.7.3 <i>Results</i>	83
	4.7.4 <i>Conclusions</i>	89
4.8	Discussion	89

Chapter 5 Is development of pecking damage in layer pullets influenced by dietary protein source? 97

5.1	Introduction	97
5.2	Methods	100
5.3	Results	103
	5.3.1 <i>Body weight</i>	103
	5.3.2 <i>Comb and wattle growth</i>	103
	5.3.3 <i>Ethogram</i>	105
	5.3.4 <i>Bird to bird pecking</i>	106
	5.3.5 <i>Pecking damage scores</i>	106
	5.3.6 <i>Egg production</i>	109
	5.3.7 <i>Plasma assays</i>	110
	5.3.8 <i>Survey samples</i>	110
5.4	Discussion	113

Chapter 6 Feather eating and its possible role in the development of pecking damage 118

6.1	Introduction	118
6.2	Experiment 1 - Feather eating and damaging pecking in juvenile pullets	119
	6.2.1 <i>Introduction</i>	119
	6.2.2 <i>Methods</i>	120
	6.2.3 <i>Results</i>	122
	6.2.4 <i>Conclusions</i>	124

Chapter 6	(continued)	
6.3	Experiment 2 - Age-related changes in feather eating and feather availability	124
	6.3.1 <i>Introduction</i>	124
	6.3.2 <i>Methods</i>	125
	6.3.3 <i>Results</i>	125
	6.3.4 <i>Comparisons between Experiments 1 and 2</i>	130
	6.3.5 <i>Conclusions</i>	130
6.4	Experiment 3 – Feather eating - a precursor of feather pecking?	130
	6.4.1 <i>Introduction</i>	130
	6.4.2 <i>Methods</i>	131
	6.4.3 <i>Results</i>	131
	6.4.4 <i>Conclusions</i>	133
6.5	Experiment 4 – Influences on feather eating in individually caged adult hens	133
	6.5.1 <i>Introduction</i>	133
	6.5.2 <i>Methods</i>	134
	Experiment 4a Are feather peckers feather eaters?	
	Experiment 4b Does the presence/absence of preen oil Affect feather eating?	
	6.5.3 <i>Results</i>	137
	Experiment 4a	137
	Experiment 4b	139
	6.5.4 <i>Conclusions</i>	141
6.6	Discussion	141
Chapter 7	General discussion	147
	Reference list	158
	Appendix A Definitions of behaviour categories	175
	Appendix B Oestradiol assay protocol and validation	176
	Appendix C Age related changes in targeting of non-aggressive pecks	178

Chapter 1

Introduction

Feather pecking is beak inflicted plumage damage caused to one bird by another. Pecking problems have been observed in domestic fowl, turkeys, pheasants, quail and ducks and can range in severity from minimal feather damage to serious feather loss, injury and mortality. Cannibalism has been a problem since intensive poultry keeping began, and there are reports of severe injurious pecking in commercial ventures as early as the 1930's (Miller and Bearse, 1937). Apart from being a serious welfare issue, feather pecking is economically costly to the egg industry through mortality and increased feed consumption associated with heat loss due to poor feather cover (Richards, 1977; Tullet et al., 1980; Peguri and Coon, 1993).

Despite a large number of studies over the last 50 years, the causation of feather pecking and cannibalism is not fully understood, and a reliable solution to the problem is still not available. Outbreaks of damaging pecking occur unpredictably, and while the problem has been associated with barren environments, it also occurs in enriched housing conditions. A greater incidence of pecking problems is associated with the use of otherwise welfare-friendly alternative production systems, and currently presents a major obstacle to the widespread adoption of non-cage systems.

Beak trimming, involving the partial amputation of the beak, is commonly carried out on an industry wide scale to reduce or prevent injurious pecking. Its effectiveness is debatable (Hughes and Mitchie, 1982; Blokhuis and Van der Haar, 1989), and the practice is considered to be a major welfare insult (FAWC, 1997). There is behavioural and neurological evidence that beak trimming can result in chronic pain (Gentle et al., 1990; Breward and Gentle, 1985) and long term adverse behavioural consequences have been demonstrated (Duncan et al., 1989). Ironically, beak trimming itself may compromise the ability to preen and therefore prove detrimental to plumage condition (Blokhuis and Van der Haar, 1989). 'Anti-pecking

devices', usually consisting of a plastic 'bit' preventing complete closure of the beak, have been designed to reduce feather pecking and cannibalism in gamebird production and rearing, where pecking problems are encountered regularly. In a study of the feasibility of anti-pecking devices for laying hens, Savory and Hetherington (1997) concluded that while damaging pecking was reduced, disruption of feeding in some individuals precluded their commercial viability.

In the first part of this introduction (Section 1.1), types of bird to bird pecking will be described, emphasising that only some bird to bird pecks contribute to pecking damage and cannibalism. In the second section (Section 1.2) main factors ('external' and 'internal') that have been shown to influence the occurrence and severity of feather pecking and cannibalism will be reviewed. The number of diverse factors that have been shown to influence feather pecking and cannibalism illustrates the complexity of the problem, which clearly involves the reaction of individual birds to their environment. Key developmental events in laying hen maturation are described in Section 1.3. Finally, descriptions of the questions addressed and approaches adopted in this project are provided in the thesis outline (Section 1.4).

1.1 Types of bird to bird pecking

Domestic fowl may peck each other in several ways, and various workers have constructed schemes defining types of bird to bird pecking (e.g. Keeling, 1995). The following definitions are adapted from Savory (1995), which contains one of the most comprehensive of such schemes. Despite routine use of the term 'aggression' in the egg industry, when referring to all forms of pecking damage, it is generally accepted that feather loss and cannibalism results from non-aggressive pecking.

1.1.1 Aggressive pecking

Aggressive pecking is related to dominance rank. These pecks may be aimed at subordinate birds by dominant individuals, or might be given during an agonistic encounter between birds of equal rank. Normally a single vigorous peck is aimed at the head, comb, neck or back, and results in the immediate withdrawal of the recipient (usually with accompanying vocalisation). Although aggressive pecks are

relatively rare, they are delivered with considerable force and therefore have the potential to cause injury.

1.1.2 *Feather pecking*

These pecks at the plumage can be described as ‘vigorous’ or ‘gentle’. Gentle pecks (sometimes called ‘allopecks’ or ‘allopreening’) are often aimed at litter particles on the plumage surface of a dustbathing recipient. They tend to be given in bouts of several pecks, are non-damaging, and are usually ignored by the recipient. Vigorous feather pecks (sometimes called ‘severe’ pecks) are also repeated persistently and can be aimed at any part of the body. These more forceful pecks can cause feather damage and may cause the recipient to withdraw eventually.

1.1.3 *Feather pulling*

Feather pulling describes a damaging behaviour where feathers are grasped in the beak and firmly pulled, often resulting in removal of the pulled feather. Damage to remaining feathers, feather loss and eventually denuded areas of skin can result. Removed feathers are usually ingested. Although feather pulling is a distinct behaviour, feather pulls are often included in the above category of ‘vigorous’ feather pecks.

1.1.4 *Tissue pecking (cannibalism)*

Denuded areas of skin exposed by feather removal are novel and attract pecking, and a wound can rapidly develop as a result of pecks at the skin. Open wounds and blood attract further attention and pecking, and the severity of injuries can accelerate in a very short time.

1.1.5 *Vent pecking*

This is considered to be the most severe form of cannibalism where pecks are aimed specifically at the cloaca, sometimes with no other pecking damage having taken place. Death of the recipient occurs rapidly through shock and loss of blood. In the worst cases, the contents of the body cavity may be removed and eaten.

It can be seen, therefore, that pecking damage results from non-aggressive vigorous feather pecks and pulls, and cannibalistic pecks. Aggressive pecks also have the potential to cause feather and tissue damage, but are much less frequent than non-aggressive pecks (Blokhuys and Arkes, 1984), and the withdrawal response of the recipient usually prevents sustained attack. Early investigations of the relationship between dominance rank and feather pecking and cannibalism were inconclusive (Hughes and Duncan, 1972; Wood-Gush and Rowland, 1973), but suggested that birds with a lower rank might be targeted. More recently, Leonard et al. (1995) showed that dominance rank was not related to any type of pecking except aggressive pecking itself, and reported that frequencies of aggressive and non-aggressive pecks were not related. Oden et al. (1999) found that the presence of males in large groups of laying hens reduced aggressive pecking but not feather pecking, indicating the different underlying nature of these types of pecking.

Gentle pecks (particularly those associated with dustbathing) are much more common than vigorous pecks or feather pulls (Leonard et al., 1995; Savory and Griffiths, 1997), and a lack of association between gentle and vigorous feather pecks has been reported (Savory and Griffiths, 1997; Kjaer and Vestergaard, 1999). This emphasises the importance of behavioural studies where observations allow different types of pecks to be distinguished.

1.2 Factors influencing pecking damage

A wide range of factors have been shown to influence feather pecking and cannibalism, which for the purposes of this introduction have been divided into two categories, 'external' and 'internal'. The topics described are not mutually exclusive, and there is inevitably some overlap between categories. In general 'external' factors are those relating to the environment, while 'internal' factors are those that are thought to be independent of the environment. Research has concentrated on external factors, with the aim of preventing or alleviating feather pecking and cannibalism by manipulation of environmental conditions. The failure of external factors alone to explain (or reliably predict) the occurrence and causation of feather pecking highlights the potential importance of internal factors.

EXTERNAL FACTORS

1.2.1 *Light*

The association between light intensity and feather pecking has been recognised for many years, and arose from casual observations that birds housed in the top tier of battery cages (where light levels are usually highest) often have poorer plumage condition than birds below. Many studies support the general rule that the incidence and severity of feather pecking in poultry increases with increasing light intensity (Hughes and Duncan, 1972; Bacon and Touchbarn, 1976; Allen and Perry, 1975; Kjaer, 1997) and as such, dimming of lights is a widely used (and effective) measure to alleviate pecking problems. There are constraints, however, on the chronic use of dim lighting as a preventative measure during rearing, as very low light intensities are known to interfere with eye development (Siopes et al., 1984).

Kjaer and Vestergaard (1999) showed that more severe pecks were delivered at high light intensity (30 lux) than at lower light intensity (3 lux), with more gentle feather pecks seen at the lower light level. Thus, light intensity not only influences numbers of feather pecks, but also their type. An examination of the effects of light intensity during rearing on pecking behaviour (Kjaer and Vestergaard, 1999) demonstrated that effects of light level during rearing could be seen to the end of the experiment (at 48 weeks), although its influence was less pronounced during the laying period than rearing period. They also found that light intensity influenced mortality through cloacal cannibalism, which was only seen after egg laying began, and suggested that increased light level could act on vent pecking by aiding visualisation of cloacal tissue.

The effect of light intensity on pecking behaviour is thought to act through the known positive association between light levels and activity (Hughes and Black, 1974; Boshouwers and Nicaise, 1987; Lewis and Morris, 1998). Kjaer (1995) pointed out that at high light levels where feather pecking is taking place, it is not clear whether feather pecking itself leads to higher activity levels (where severe pecks disturb the recipient, causing it to move) or vice versa. However, exposure to light is known to induce behavioural and physiological arousal in rats (Sasaki et al., 1996), and higher activity levels have been observed under increased light intensities

(in the absence of feather pecking) in domestic fowl (Boshouwers and Nicaise, 1987; Boshouwers and Nicaise, 1993).

In contrast to the results of most studies, Martin (1989) reported less feather pecking and more ground pecking at 500 lux compared to 50 lux, and suggested that light intensity could act directly on pecking behaviour by enabling birds to visualise and peck accurately in their environment. Similarly, Johnsen et al. (1998) found no adverse effects on feather pecking of providing a strongly lit area (1000 lux) for dustbathing during rearing, suggesting that high light intensities can be applied safely under particular conditions. The very high light intensities used in these studies make comparisons with those described above (which compared light intensities under 30 lux) difficult, but may indicate that very high or very low light intensities are least likely to promote feather pecking.

Apart from light intensity, light wavelength (colour) is another possible influence on behaviour. Red filters have been employed to reduce pecking problems, and probably act through decreasing light intensity (and therefore activity) and by reducing the visibility of blood and wounds (Wells, 1971; Appleby et al., 1992). Schumaier et al. (1968) found more feather pecking in white leghorns kept under green or white light than under red light. Light source may also have an effect on pecking; physical activity has been found to be higher in birds kept under fluorescent light than in incandescent light of the same intensity (Boshouwers and Nicaise, 1993). Chickens perceive some types of fluorescent lighting as discontinuous (flickering), but this does not appear to be aversive (Lewis and Morris, 1998). On the contrary, Widowski et al. (1992) showed that hens preferred fluorescent over incandescent lighting.

1.2.2 *Floor substrate*

The causation of feather pecking has been explained in terms of redirected feeding behaviour (Hoffmeyer, 1969), or more recently, redirected ground pecking, either in foraging or dustbathing contexts. It is generally accepted that ground pecking and feather pecking share common causal factors, but two hypotheses have arisen through independent research programmes to explain how the two behaviours might be linked.

The first school of thought proposes that feather damage and pecking problems arise from redirected ground pecking under the control of foraging motivation (e.g. Blokhuis, 1986). It is argued that ground pecking in poultry is part of the foraging system, and as such redirected ground pecks (i.e. to other birds) are under the control of the feeding system. Blokhuis (1986) showed that ground pecking increased after feeding in birds with access to litter, while pecking at conspecifics increased after feeding in birds without access to litter. Blokhuis (1989) also demonstrated that a change in floor type from litter to a slatted floor decreased ground pecking and increased feather pecking in 6-week-old pullets. These studies suggest that it is important to provide poultry with suitable floor substrates (as incentives for ground pecking) in order to reduce or prevent feather pecking.

The second hypothesis argues that the relationship between feather pecking and ground pecking arises through dustbathing, where feathers come to be perceived as a dustbathing substrate (Vestergaard and Lisborg, 1993). It is proposed that this can be prevented if a suitable dustbathing substrate is provided at an early age. When given a choice, inexperienced chicks showed a marked preference for sand as a substrate for dustbathing, but exhibited no preference between wood shavings and feathers (Sanotra et al., 1995). This led the authors to suggest that rearing chicks on wood shavings introduces a pathological risk of feather pecking developing, unless a suitably attractive dustbathing substrate is also provided. If birds do become imprinted on feathers as dustbathing substrate, it is argued that difficulty initiating dustbathing (during the appetitive phase where the substrate is pecked) leads to feather pecking (Vestergaard et al., 1993). Norgaard-Nielsen (1997) tested this notion, but found it impossible to separate pecks related to dustbathing from pecks belonging to other motivational systems. In support of the dustbathing hypothesis, Leonard et al. (1995) found that feather pecking increased when birds showed intention to dustbathe and decreased when dustbathing ended. Vestergaard et al. (1997) suggested that the lack of a suitable substrate to perform dustbathing is a stressor, which might promote feather pecking and cannibalism.

It is likely that the foraging/dustbathing hypotheses are not mutually exclusive, and it has been suggested that both feeding and dustbathing may act as releasers for pecking at non-food objects (Savory, 1995). Whatever the nature of the

association between ground pecking and feather pecking, many studies have demonstrated that the lack of a suitable floor type (ie pecking substrate) can encourage feather pecking (Simonsen et al., 1980; Blokhuis, 1991).

Savory and Mann (1999) investigated whether litter particles on the plumage surface (as a result of dustbathing) could have a direct effect on feather pecking. They proposed that the level of contrast between plumage colour and litter colour could affect the propensity to be pecked due to litter particles on the plumage having a greater stimulus value. There was no effect of litter substrate on pecking damage scores, although behaviour observations showed that more pecks from light coloured birds were directed at light litter particles on dark birds. It is possible that the stimulus value of light particles on dark plumage was lower for dark birds, which would have encountered this stimulus while preening.

1.2.3 *Stocking density/group size*

A common explanation for the occurrence of feather pecking and cannibalism in the past was that it was a detrimental side-effect of 'overcrowding' (Sanctuary, 1954), and it is obvious that the close proximity of a flock-mate is required for feather pecking to occur. Modern egg production systems are characterised by high stocking densities, and in the case of non-cage 'alternative' systems, large group sizes. Stocking density (i.e. birds per unit area) and group size (i.e. number of birds with physical access to each other) could affect feather pecking separately or collectively, but many studies have failed to control for these separate factors.

Experiments using small groups (usually in cages at high density), comparing group sizes less than ten, have implicated increasing group size in promoting feather pecking and cannibalism more often than high density (Hughes and Duncan, 1972; Hughes and Black, 1974; Allen and Perry, 1975; Kivimae, 1976; Quart and Adams, 1982). Other studies have reported negative effects of high stocking density on plumage condition (Simonsen et al., 1980; Appleby et al., 1988). Savory et al. (1999) carried out an experiment aiming to separate the effects of group size and density, comparing two group sizes (10 and 20 birds) at low, intermediate and high densities. The results suggested group size - density interactions, with high density having a greater effect in the larger group size.

It is possible that group size could alter pecking behavior by affecting social recognition, and it has been suggested that the ‘unnaturally’ large group birds encounter in alternative housing systems may promote pecking damage (Appleby, 1993; Abrahamsson et al., 1995). It seems more likely, however, that group size effects arise simply because the larger the group size, the more potential victims that are available to feather pecking birds (Keeling, 1994). This ‘access effect’ confounds studies of group size, especially those where pecking behaviour is not observed and feather pecking is evaluated only by plumage damage scores. Stocking density, on the other hand, could affect feather pecking directly by bringing birds into closer physical contact with each other and possibly by suppressing ground pecking in crowded conditions.

1.2.4 *Diet*

Among the first studies investigating the causation of injurious pecking were those relating to dietary factors, presumably under the assumption that birds exhibiting cannibalistic behaviour were receiving inadequate amounts of some nutrient. Miller and Bearnse (1937) found that inclusion of ground oats in the diet reduced feather pecking and cannibalism, while birds fed on corn as the sole cereal source exhibited high levels of cannibalism. He concluded that corn based diets encouraged injurious pecking because they were deficient in some beneficial ‘factor’. Further work (Miller and Bearnse, 1938; Bearnse et al., 1940) investigated the cannibalism preventing properties of oats, and concluded that it was the fibre content of the oat hulls, rather than some mineral or other nutrient, that was responsible. Observations that ‘chickens with the feather pulling vice’ regularly ate the feathers they removed, suggested to Willimon and Morgan (1953) that there was a relationship between feather pecking and the mineral content of feathers. However, supplementation of the diet with various minerals failed to indicate any consistent effect. Amino acid deficiencies have also been investigated as a possible cause of feather pecking. Neal (1956) reported that supplementation of the diet with methionine caused a marked alleviation of feather pecking and cannibalism during an outbreak, with pecking problems resuming when the supplementation ceased. Bessei (1978) reported that hens fed a sodium deficient diet exhibited feather pecking and

increased activity levels, but Hughes and Whitehead (1974) failed to find a consistent effect of sodium deprivation on the occurrence of feather pecking. Savory et al. (1999) showed suppression of pecking damage in growing bantams fed diets supplemented with 20g/kg tryptophan. It was suggested that the sedative-like properties of tryptophan were responsible for the reduced pecking, but at current prices, such levels of tryptophan inclusion would not be commercially viable for layer flocks.

The occurrence of feather pecking and cannibalism, therefore, has been attributed to deficiencies in various minerals and amino acids, but it is difficult to relate the results of early studies to modern hybrids and production systems. The failure of subsequent workers to replicate earlier findings reflects the unpredictability of the problem. While modern diets are much improved, the nutritional demands of today's highly selected egg producing lines are constantly changing (as are the range of ingredients being utilised for layer diets) and it is still possible that dietary factors may contribute to the development of pecking problems. Nevertheless, effects of diet on feather pecking and cannibalism in modern layer strains remain one of the least investigated aspects of the problem.

Recently, there has been speculation that feather pecking and cannibalism may be related to dietary protein source. Reports of outbreaks of feather pecking and cannibalism after changes in the diet from mainly animal to mainly plant protein (Curtis and Marsh, 1992) support anecdotal evidence from egg producers that the presence of animal protein in layer diets (especially fishmeal) is effective in preventing or even halting outbreaks of damaging pecking. The ban on the inclusion of meat and bone meal in animal feeds imposed in the UK in the wake of the bovine spongiform encephalopathy outbreak in cattle, have resulted in major alterations in diet formulation for poultry. In conjunction with the high cost of fishmeal this has led to the widespread use of layer diets which rely exclusively on plant protein sources, and it has been suggested that this trend is causally related to higher levels of feather pecking and cannibalism observed in recent years (e.g. FAWC, 1997). It has been assumed that there is something beneficial present in animal protein sources, but it is equally possible that any effects could be due to detrimental substances in plant protein sources. Soyabeans, commonly used as a plant protein

source, are a rich source of phytoestrogens (Sheehan, 1993; Knight and Eden, 1995), and it is conceivable that these biologically active compounds could affect behaviour.

The effect of protein level on the incidence of feather pecking has been examined, with lower protein diets being associated with increased pecking damage (Cain et al., 1984; Ambrosen and Petersen, 1997), but only one study investigated the effects of dietary protein source. Savory et al. (1999) fed growing bantams isonitrogenous diets containing either plant, mainly animal or mainly semipurified protein. No difference in pecking damage was observed between treatments, but it is possible that any effects of phytoestrogens could depend on stage of sexual maturation and therefore did not influence the behaviour of the juvenile bantams in this study. Thus, any effect of animal or plant protein source on feather pecking and cannibalism in layer pullets remains to be confirmed by experiment.

Apart from any nutritional considerations, it is known that food form (food particle size) can influence the incidence of pecking problems. Pelleted diets are associated with more pecking damage than mash (Heywang and Morgan, 1944; Bearnse et al., 1949; Calet, 1965; Lindberg and Nicol, 1994) and consequently commercial flocks are routinely fed on mash, even though pelleted diets promote faster growth (Heywang and Morgan, 1944; Lanson and Smyth, 1955; Calet, 1965). Savory et al. (1999), working on bantams, confirmed that pecking damage was greater with pelleted diets, but found no significant difference between the low pecking levels observed with mash and a diluted mash treatment. A likely explanation for the food form effect is that more time and pecking (at food) must be devoted to eating mash compared to pellets (Lanson and Smyth, 1955; Jensen et al., 1962), leaving a smaller portion of the time budget available for bird to bird pecking.

1.2.5 *Environmental enrichment*

Environmental enrichment devices aim to increase the complexity of the environment, and various objects and 'toys' have been utilised for this purpose experimentally. The benefits of providing environmental enrichment on production traits such as egg production have been demonstrated (Bell et al., 1998), and Gvoryahu et al. (1994) found a significant reduction in aggressive pecking when

caged birds were provided with coloured hanging enrichment objects. Yasutomi and Adachi (1989) reported reduced cannibalism with the introduction of 'playthings'. These effects were attributed to a reduction in 'boredom' and redirection of pecks away from other birds. However, there is a risk that birds will become habituated to environmental enrichment devices (Sherwin, 1993), and this, coupled with the practicalities and costs of installation, have prevented such measures from being applied on a commercial scale.

1.2.6 *Production System*

The extent to which any production system promotes or prevents feather pecking and cannibalism will presumably depend on the effects of the various environmental factors already discussed acting together. Hence, any production system's effects on the incidence of pecking problems may be explained in terms of light levels, group size, stocking density or access to pecking substrate. Egg production systems can be defined as intensive or extensive; battery cage systems are considered intensive, while free range, deep litter, perchery (barn) and aviary systems are considered extensive. At present in the UK, 80% of laying hens are housed in cages, with only 20% in non-cage systems (free range 15%, barn 5%, British Egg Information Service, 1998). Recently, there has been a trend towards extensive systems as a result of public concern for the welfare of birds in cages. In many respects, the welfare of birds in non-cage systems is better than those in cages, but high mortality through serious pecking problems has been encountered (Abrahamsson and Tauson, 1995; Abrahamsson et al., 1996), and injurious pecking is considered to be the main obstacle in the practical application of alternative non-cage systems (Appleby, 1993). Nevertheless, several authors have reported more feather pecking in cages than in pens with litter (Hughes and Duncan, 1972; Appleby et al., 1988). This increased pecking damage is likely to be due to a combination of high stocking density, lack of pecking substrate and a barren environment. In addition, it must be noted that feather damage and loss can occur in cages through abrasion (Hughes, 1980). Importantly, however, overall flock mortality through pecking damage is lower in cages, since the problem is contained in small groups and peckers have access to fewer victims (Keeling, 1994; Savory, 1995). In non-cage

systems with large group sizes, such pecking individuals have the potential to inflict much more damage.

Modified cages have been developed which provide improved environments for laying hens, and these may be preferable to non-cage systems by containing damaging pecking (Appleby, 1993; Appleby et al., 1993; Tauson, 1998). However, there is some evidence that changing the shape of cages and provision of perches could worsen pecking problems. Moinard et al. (1998) reported increased pecking problems in heightened cages allowing more light in, and proposed that increased visibility of the cloaca of perching birds encouraged vent pecking. Hughes and Black (1976) also noted that pecking damage was worse in deeper cages. Modified cages are not in commercial use in the UK, but these considerations would need to be taken into account in any evaluation of their welfare benefits and economic viability.

INTERNAL FACTORS

1.2.7 Genetic variation

Obvious strain differences in the incidence of feather pecking and cannibalism (Hughes and Duncan, 1972; Abrahamsson and Tauson, 1995; Savory and Mann, 1997) demonstrate that propensity to feather peck has an inherited component. Hughes and Duncan (1972) found that types of damaging pecking varied between strains, with feather loss more common in one strain, and cannibalism more common in another. More recently, comparison of genetic lines found to exhibit 'high' or 'low' feather pecking have been a useful source of information about feather pecking and related traits. Blokhuis and Beuving (1993) compared two such lines of White Leghorn hens exhibiting 'high' or 'low' levels of feather pecking. They found that birds belonging to the high pecking strain spent less time ground pecking and more time pecking at objects than their low feather pecking counterparts, suggesting a genetic effect on the expression of pecking, rather than simply propensity to peck.

In addition to strain differences, it has become clear there is individual genetic variation affecting propensity to peck within flocks, where a small number of birds can account for most of the pecking damage (Keeling, 1994; Savory, 1995). Savory and Griffiths (1997) reported individual variation in rates of giving and

receiving feather pecks in bantams, demonstrating that only a few birds were giving and receiving many pecks/pulls. Wechsler et al. (1998) examined individual variation in the behaviour of juvenile hens, and found that while 83% of birds engaged in some type of bird to bird pecking, only 39% were defined as high peckers (by having a pecking rate more than twice the average for the group). These high peckers also delivered more severe pecks and pulls than birds with low pecking rates. Hughes (1985) introduced the concept of 'primary' feather peckers with an innate (genetic) propensity to peck and 'secondary' opportunistic peckers who may be attracted to wounds and exacerbate the situation.

The idea that high peckers might have different 'personalities' led to investigations looking for behavioural correlates of individual variation in propensity to feather peck. While feather pecking and cannibalistic birds have been shown to be more active (Keeling and Jensen, 1995; Savory and Griffiths, 1997; Savory and Mann, 1997), no clear associations with comfort, social, or aggressive behaviours have been found.

As well as a genetic tendency to feather peck, it has also been suggested that some individuals might have a genetic predisposition to be pecked (Cuthbertson, 1978). However, receiving feather pecks has since been found to have zero heritability (Kjaer and Sorensen, 1997), supporting observations that feather pecks tend to be targeted indiscriminately (Leonard et al., 1995; Wechsler et al., 1998).

It has been suggested that genetic selection of modern hybrids for increased egg production might have inadvertently selected for increased feather pecking and cannibalism (Hughes and Duncan, 1972), and Bhagwat and Craig (1977) found a relationship between age at first egg and social dominance. Kjaer and Sorensen (1997) found a consistent negative genetic correlation between body weight and 'performing feather pecking', and concluded that selection for smaller body size as part of commercial breeding programmes might have contributed to feather pecking problems in laying hens. Feather pecking behaviour may have been selected for 'naturally' since peckees would be more likely to be removed from a population as a result of mortality than birds with a higher tendency to peck (Hughes and Duncan, 1972). Choice of breeding birds with the best plumage may also have contributed to inadvertent selection for peckers (Cuthbertson, 1980).

Given that studies of genetic variability of performing feather pecking behaviour have given rise to heritability estimates of low to moderate size (Cuthbertson, 1980; Kjaer and Sorensen, 1997), it should be possible to select against injurious pecking. This was shown to be feasible by Craig and Muir (1993), who produced a reduction in beak inflicted injuries after only two generations of selection against feather pecking, and as mentioned previously, genetic lines of white leghorns exhibiting 'high' or 'low' feather pecking have been studied. For selection against feather pecking to be successfully achieved on a commercial scale, appropriate selection traits must be identified. In the past, selection has been based on direct observation (Keeling and Wilhelmson, 1997), but this approach is time consuming and exposes birds to the risk of injury. Bessei et al. (1997) developed an automatic device to measure pecks at a bunch of feathers, allowing gentle and vigorous pecks/pulls to be distinguished. It is possible that such a technique could be adapted commercially to screen large numbers of birds and identify individual differences in propensity to feather peck as a basis for selection. This approach has potential for reducing feather pecking and cannibalism in commercial strains, but the apparent multi-factorial causation of feather pecking suggests that complete eradication of the problem by genetic selection may not be possible.

1.2.8 *Age/development*

Several investigations of age-related changes in feather pecking have been carried out, aiming to identify possible precursors of the behaviour and improve our ability to predict outbreaks of damaging pecking. Distinct phases in the development of feather pecking appear to be present. An early peak in damaging pecking between 4 and 10 weeks of age has been observed, followed by a decline in pecking in the latter part of the rearing period (Hughes and Duncan, 1972; Hughes, 1973; Kjaer and Sorensen, 1997). This is followed by a general increase in feather pecking (and in some cases appearance of vent pecking) at sexual maturity (see Section 1.2.9).

There is growing evidence that early experience can have a profound effect on feather pecking behaviour in later life, and it is thought that past experience is crucial in the validation of such stimuli as incentives for pecking (Blokhuys and Van

der Haar, 1989; Blockhuis and Van der Haar, 1992). It has been suggested that there might be a sensitive period during the first 10 days of life where exposure to sufficient pecking substrate might affect later behaviour (Sanotra et al., 1995). This view was supported by a study by Huber-Eicher and Wechsler (1997), who found that chicks with access to sand from the first day of life feather pecked less than chicks with access to sand from day 10 onwards.

Most rearing studies have investigated effects of floor substrate and have tested predictions arising from the foraging/dustbathing hypotheses outlined previously (see 1.1.2). Norgaard-Nielsen et al. (1993) showed that rearing substrate had a significant influence on plumage condition, with access to sand and peat being most beneficial. In a comparison of rearing birds on slatted floors or litter, Blokhuis and Arkes (1984) found that feather pecking only developed in groups reared on slatted floors, while birds reared on litter spent more time ground pecking and exhibited only gentle, non-damaging pecking. Similarly, Johnsen et al. (1998) examined effects of floor type during rearing, rearing chicks on sand and straw, straw alone or wire. Chicks reared on sand and straw did not develop feather pecking, those reared on straw alone developed mild pecking damage, and birds on wire developed severe feather pecking and cannibalism. The authors of this study also noted that if feather pecking developed early in chicks, attempts to alleviate the problem by enriching the environment in adulthood were usually unsuccessful.

Such rearing studies provide convincing evidence that hens' perception of stimuli for pecking is influenced by experience during rearing, but the results could be argued to support both the foraging and dustbathing hypothesis for the causation of feather pecking and cannibalism. Thus, provision of pecking substrate during early development could affect the incentive value of the floor as a foraging substrate and/or the perception of substrate for dustbathing. Huber-Eicher and Wechsler (1997) carried out an experiment attempting to test the predictions of both theories, in a rearing experiment examining behavioural development to seven weeks of age. Their study showed that providing chicks with sand and straw prevented the development of feather pecking, but provision of sand alone did not. No significant differences in dustbathing were observed between treatments, but birds provided with straw spent more time foraging and feather pecked less. It was therefore

concluded that provision of an appropriate substrate for dustbathing did not prevent feather pecking, and that housing conditions promoting foraging behaviour in the first weeks of life (by increasing the stimulus value of the floor substrate) could reduce or prevent damaging pecking. Therefore, this study strongly supports the theory that feather pecking is misdirected foraging behaviour.

Feather pecking and cannibalism can still occur when birds are reared on litter (Norgaard-Nielsen et al., 1993), and a theory has been proposed for the development of damaging pecking in these cases. Savory and Mann (1997) reported that an age-related decline in ground directed foraging pecking coincided with increase in preening and dustbathing. They suggested that this might encourage redirection of pecking towards feathers and lead to damaging pecking.

Roden and Wechsler (1998) investigated whether presence or absence of a hen had any effect on the development of feather pecking in young chicks. The presence of a hen during rearing did affect many aspects of chick behaviour, but had no effect on feather pecking behaviour.

1.2.9 *Hormonal state*

Several authors have noted that feather pecking begins or worsens around the onset of lay (Dickerson and Kashyap, 1961; Hughes and Duncan, 1972; Hughes, 1973; Allen and Perry, 1975; Blokhuis, 1991; Norgaard-Nielsen et al., 1993; Bilcik and Keeling, 1999). Such observations, in addition to reports that female pullets generally feather peck more than males, indicate a possible hormonal influence on the development of feather pecking and cannibalism. Only one study (Hughes, 1973) examined the effect of hormonal state on feather pecking and cannibalism experimentally. In this experiment, the increase in feather pecking normally associated with the onset of lay was advanced in juvenile pullets treated chronically with hormone implants. Treatment with oestradiol or progesterone alone had little effect, while a combination treatment caused a large increase in feather pecking. Testosterone implants prevented the 'normal' increase seen at the onset of lay. It is well known that the increased synthesis of progesterone receptors depends on prior exposure to oestradiol (Kawashima et al., 1979; Kamiyoshi et al., 1992; Kawashima et al., 1992). If progesterone plays a central role in the aetiology of feather pecking

then oestrogen may exert its effects on behaviour through changes in progesterone receptor regulation. This may explain why the only treatment found by Hughes (1973) to advance pecking damage was the oestradiol and progesterone combination. It is possible that plasma levels of oestradiol alone could influence feather pecking by increasing activity. Horne and Wood-Gush (1970) observed increased exploratory behaviour in hens treated with oestrogen, and plasma oestrogen and activity levels have been linked in mammals (Cushing et al., 1995).

There is a paucity of information relating physiological parameters to feather pecking, especially in individuals from the same strain. Korte et al. (1997) measured the corticosterone response to restraint in individuals from genetic lines selected for 'high' or 'low' feather pecking. They found that birds from the high feather pecking line were 'active copers' exhibiting a lower corticosterone response to stress than low pecking line birds. Increased heart rate during manual restraint in chicks of the same high pecking line was also demonstrated by Korte et al. (1999).

Vent (cloaca) pecking, a specialised and particularly destructive type of cannibalism, is usually only seen at and after the onset of lay. This suggests that either this type of cannibalism is directly related to hormonal state, or that some aspect of the oviposition process increases the attractiveness of the cloaca as a stimulus for pecking. It has been suggested that the appearance of the vent might be altered immediately after an egg is laid (by the red lining of the cloaca being visible, for example), attracting pecking and leading to cannibalism (Turnhill and Feltwell, 1952; Sanctuary, 1954). Given the known attractiveness of blood and wounds, any partial prolapse or abnormal eversion of the vent (which would be more likely in the first weeks of lay) could also initiate vent pecking (Savory, 1995). Gunnarsson et al. (1999) argued that if pecking is directed at the vent immediately after oviposition, a correlation between the incidence of floor laying (where flockmates are present) and cloacal cannibalism would be expected. However, in their study of commercial flocks, no such correlation was found.

Whilst Hughes (1973) provided convincing evidence for a role of gonadal hormones in the development of damaging pecking, the exact nature of any hormonal influence remains unclear since neither behaviour or plasma hormone levels were measured. It should also be noted that the numerous studies reporting the presence

of feather pecking in juvenile birds (e.g. Blokhuis and Arkes, 1984; Huber-Eicher and Wechsler, 1997) demonstrate that other (probably non-hormonal) causative factors must be involved at earlier stages of development.

1.2.10 *Fear*

There is some evidence of a positive relationship between fear and pecking damage. Hughes and Duncan (1972) showed that fear levels were higher in cages where feather pecking and cannibalism occurred, but they could not ascertain whether fearfulness was a cause of pecking damage or a response to it. More pecking damage was reported in 'nervous' birds by Ouart and Adams (1982), and individual Red Jungle Fowl characterised as 'feather peckers' were found to be more fearful by Vestergaard et al. (1993). Keeling and Jensen (1995) also reported that cannibalistic hens were more fearful of an approaching predator than controls. Comparisons made by Blokhuis and Beuving (1993) of high (HP) and low feather pecking (LP) genetic lines showed that birds of the high pecking line had longer tonic immobility (TI) response times, which are thought to indicate higher fear states (Jones, 1996). Working on chicks of the same HP and LP lines however, Jones et al. (1995) failed to demonstrate the same difference in TI response, although a difference in open field behaviour (another measure of fear state) was observed. These studies suggest an association between fear and injurious pecking, but do not demonstrate direct cause and effect or show whether peckers or pecked birds are more fearful. It has been suggested that the different open field responses observed in the HP and LP lines reflect differences in underlying sociality, rather than fearfulness (Jones, 1996).

Extreme fear reactions, such as panic and hysteria, have also been associated with feather pecking and cannibalism, but the nature of this association is unclear. Mills and Faure (1990) suggested that pecking damage was a possible causative factor in the development of hysteria reactions in layer flocks, but conceded that both problems are more likely to be related to particular housing conditions than to each other. It is possible that feather loss and wounds sustained through trampling and clawing during an episode of hysteria could become targets for subsequent pecking, and this could explain the apparent connection.

1.3 Sexual maturation in laying hens: hormonal and behavioural changes

Modern egg laying strains have been selected over several years for increased egg number, and this has been achieved partly by reducing the age at which sexual maturity occurs and egg production commences (Appleby et al., 1992). As a consequence, the onset of ovarian activity and associated hormonal changes occur much earlier in modern commercial birds than in their predecessors. Much of the classical literature describes the time course of the endocrinological features of the onset of lay in these older lines. Whilst the nature and pattern of gonadotrophin and steroid secretions are essentially similar in modern birds, the timing of these events has clearly altered in response to genetic selection pressure. As sexual maturity approaches, characteristic changes occur in the plasma concentrations of oestradiol, progesterone and testosterone (Figure 1.1). A temporary peak in plasma oestradiol concentration is observed around four weeks before egg laying begins (Senior, 1974a), while plasma progesterone concentration increases rapidly in the week before the first eggs are laid, and remains elevated while laying continues (Williams and Sharp, 1977). Plasma testosterone concentration increases gradually throughout the growing period (Itoh et al. 1988).

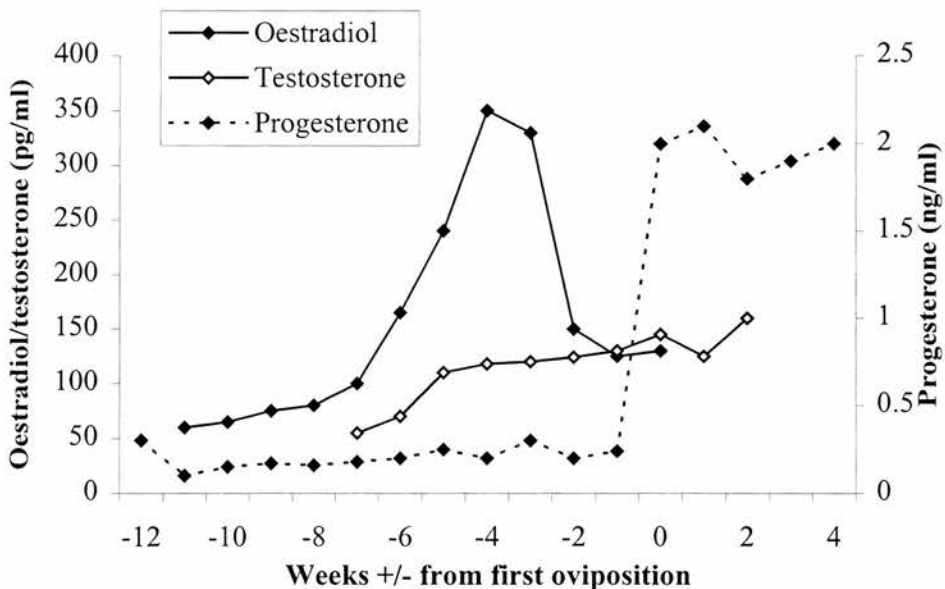


Figure 1.1 Plasma concentrations of oestradiol, progesterone and testosterone in relation to the onset of lay in pullets. Redrawn from Etches (1996).

Although several studies have examined the development of bird to bird pecking (see Section 1.2.8), few of these have reported age related changes in other behaviours. Savory and Mann (1997) examined behavioural development in pen-housed pullets, and reported age-related changes in several types of behaviour. Time spent feeding, sitting and pacing declined after the first four weeks of life, while litter directed behaviour was highest in the growing period, between 5 and 12 weeks. After the onset of lay, marked increases were observed in time spent preening and dustbathing.

1.4 Thesis outline

The literature review above illustrates that while the influence of some (mostly external) factors in the development of feather pecking have been well described, others have received little attention. One of the most striking omissions is the lack of physiological data relating to feather pecking, particularly the physiological basis of developmental changes in the nature and extent of bird to bird pecking and individual differences between birds identified as peckers and non-peckers. The aim of this project was to address these issues, with particular attention to changes in behaviour and physiological state associated with sexual maturity in layer pullets. In the light of evidence for the involvement of oestradiol and progesterone (Hughes, 1973), the role of gonadal hormones in the development of damaging pecking was investigated.

Chapter 3 describes a long-term study of behavioural and hormonal development, which examined age-related changes in behaviour in layer pullets, to 26 weeks of age, in relation to plasma concentrations of oestradiol, progesterone and testosterone.

The circumstantial link between changes in gonadal hormonal state and pecking behaviour was investigated in the experiments described in Chapter 4. The overall aim was to manipulate hormonal state to determine whether the temporal relationship between changes in bird to bird pecking behaviour and hormonal state was causal in nature. The intended hormonal manipulation (the advancement of sexual maturation in juvenile pullets) proved difficult to achieve, necessitating a series of experiments investigating the effectiveness of various routes of chronic

gonadal hormone administration. In the absence of a reliable method of long term agonist administration, a final experiment investigated the effects of acute antagonist (tamoxifen) administration on pecking at a novel pecking device in hens identified as 'peckers' or 'non-peckers'.

In Chapter 5, anecdotal claims of an association between the increased use of plant protein sources in laying hen diets and increased incidence of pecking damage were examined. A long-term behavioural development study compared the behaviour and hormonal state of pullets fed diets based on animal (fishmeal) or plant (soyabean meal) protein. Possible behavioural and physiological effects of oestrogenic compounds present in soyabean meal (phytoestrogens) were investigated.

Throughout the project, evidence from various experiments implicated feather eating, a common behaviour in adult and juvenile pullets, in the development of damaging pecking. In Chapter 6, experiments investigating the relationship between feather eating and feather pecking in juvenile pullets along with age related changes in feather eating behaviour are described. The extent to which feather eating is a precursor of feather pecking was also examined. In individual birds, it was determined whether feather peckers were also feather eaters, and whether the presence or absence of preen oil on the feather surface affected feather attractiveness.

Chapter 7 provides a general discussion of findings presented in this thesis in relation to existing knowledge about the development of feather pecking and cannibalism.

Definitions of the behaviour categories used during systematic observations are given in Appendix A. Appendix B is a full description of the assay used to measure plasma oestradiol and its validation. Additional information describing age related changes in the targeting of bird to bird pecks is provided in Appendix C.

Chapter 2

General materials and methods

2.1 Subjects

Female domestic fowl (*Gallus gallus domesticus*) of a commercial laying strain (ISA Brown) were used in the studies reported in this thesis. ISA Brown hens (originally derived from Rhode Island Red × Rhode Island White) are the biggest selling brown egg layers worldwide and are widely used in UK egg production. Peak production (95%) is reached at 25 weeks of age and production is maintained above 90% for 20 weeks (ISA Brown Management Guide, 1996. Hubbard ISA, Worldwide Layer Operations, France).

Brown Leghorn pullets were used in one study (Chapter 4, Experiment 1b). A flock of this non-commercial strain is maintained at Roslin Institute for experimental purposes, and chicks are hatched on site.

2.2 Husbandry

This section describes husbandry procedures common to experiments where birds were reared in pens. In studies where birds were housed in cages, details of methodology are described in the appropriate chapters.

2.2.1 Housing and rearing

The same set of 12 adjacent small pens was used in all experiments. The pens were in a line along one wall of a large windowless room and each measured 1.6m x 1.2m. The pen walls were solid (wood or metal) to a height of 112cm, above which they consisted of wire mesh (aperture 5cm) to the ceiling allowing light penetration and bird observation. Therefore, birds in adjacent pens were physically and visually isolated from each other but could hear vocalisations of neighbours. A door at the front of each pen allowed access, with a doorsill (height 40cm) to retain litter.

Each pen contained one red plastic bell-type automatic drinker and one metal food hopper, providing water and food *ad libitum*. Food was presented in the form of pellets (3mm diameter). Unless stated otherwise, all groups received starter diet (205

g/kg crude protein (CP), 11.5 MJ/kg metabolisable energy (ME)) for the first 6 weeks of life, a grower diet (164 g/kg CP, 11.8 MJ/kg ME) from 7 to 16 weeks, and a layer diet (158g/kg CP, 11.2 MJ/kg ME) from 17 weeks. Mash was provided with pellets for the first few days of life in wide (38cm diameter) plastic trays.

A layer of clean wood shavings (approximately 10cm deep) was provided in each pen at the start of each experiment, which was not renewed. In later experiments (Chapter 4, Experiment 1a; Chapter 5), the litter was weighed (9kg per pen), to ensure that equal amounts were present in each pen.

Light was provided from eight tungsten ceiling lights containing 100W bulbs and an additional 100W light source present in each pen. Light intensity at floor level varied between pens (84 to 144 lux, overall mean 116 lux) because of the spacing of ceiling lights. These relatively high light intensities (compared to commercial conditions) were intended to increase the likelihood of bird to bird pecking and aid behaviour observations. In the behavioural development studies, where birds were reared in pens from day old to sexual maturity, the light regime was 10h L:14h D until 14 weeks, after which the light period was increased by one hour in each of the next four weeks until the photoperiod was 14h at 18 weeks. Daily temperatures varied depending on the time of year, and ranged from 10 to 31°C (mean 22°C). A dull emitter heater was placed in each pen to provide supplementary heating for chicks until six weeks of age.

2.2.2 *Bird identification*

Although chicks were assigned uniquely numbered wing tags at one day-old, birds were also fitted with a coloured plastic leg band on each leg to allow easy identification without handling during behaviour observations. Leg bands of three sizes were used (6, 10 and 15 mm diameter), which were changed as required by leg growth. Twelve unique leg band colour codes were used which were maintained throughout all experiments using groups of 12 birds (green, red, black, yellow, blue, pink, orange, purple, black/yellow, red/green, blue/pink, and yellow/red). Up to 3 weeks of age, the chicks' leg bands were often obscured by litter, or because they were sitting. Therefore, a series of black dots on the top of the head (made with permanent marker pen) corresponding to the leg band colour code (Figure 2.1) were used to aid identification. Individual features of plumage colour and tail shape aided

identification of older birds when leg bands were not visible (e.g. while a bird was sitting or dustbathing).

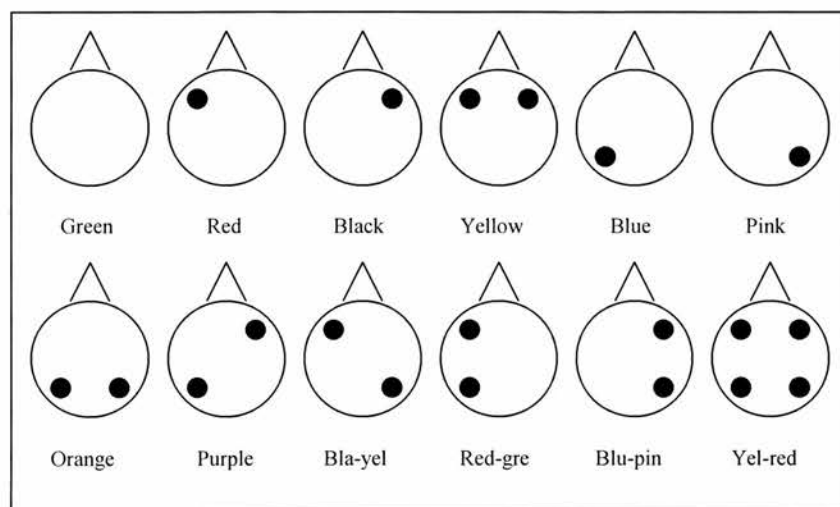


Figure 2.1 Head dots corresponding to leg band colour codes for chick identification

2.3 Growth measurements

2.3.1 *Bodyweight*

Individual bodyweights were obtained using a top-loading balance (Ohaus Explorer) with an animal-weighing programme (providing an average of 16 measurements).

2.3.2 *Comb and wattle growth*

At the time of weighing, the maximal length and height of the comb and left wattle was measured to the nearest mm using a 8cm long strip of card marked at 1mm intervals. Length and height measurements were multiplied to give an estimation of area and hence a measure of comb and wattle growth.

2.4 Behaviour

All behavioural recordings were made by direct observation by the author, from a seated position outside the pen.

2.4.1 *Ethogram*

Ethogram observations were generally carried out in alternate weeks. 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 KEYBEHAVIOUR package (Deag, 1994). Observations of each group consisted of 6 scans, recording a single ‘on the dot’ observation (Martin and Bateson, 1986) of behaviour for every bird (in a fixed order, prompted on the computer screen). Activities were recorded as letter codes corresponding to 12 mutually exclusive categories. These were sitting (only), standing (only), walking, pecking litter, feeding (or feeder directed), drinking (or drinker directed), preening standing, preening sitting, dustbathing, scratching, pecking another bird non-aggressively, and pecking another bird aggressively (definitions of these behaviours are given in Appendix A). Therefore, 72 observations were obtained per group per session (6 scans x 12 birds). The KEYBEHAVIOUR data files were interpreted using a spreadsheet (Excel 97) after transferral to a PC.

2.4.2 *Bird to bird pecking*

Detailed observations of bird to bird pecking were generally carried out weekly. Pens were observed in random order, twice weekly (one morning and one afternoon session). Every bird in each pen (identified by unique coloured leg rings) was observed individually for one minute, each pen being observed for a total of 12 minutes per session. During each ‘focal’ observation, every peck and feather pull given or received by the subject was recorded on a checksheet. Pecks were defined as either non-aggressive (subdivided into ‘gentle’ or ‘vigorous’ in some experiments) or aggressive (see Appendix A for definitions). Non-aggressive pecks were recorded according to the area of the body targeted; wing (or wing band), beak, back, head (including comb), tail, vent or neck/breast. For all peck types and feather pulls the behaviour exhibited by the recipient immediately prior to being pecked was also noted on the checksheet, using the ethogram activity letter codes described above. Feather eating events and the source of the feathers eaten were also recorded. Data from morning and afternoon sessions were combined to give total counts for each pen in each week. Because of the known individual variation in pecking behaviour, it was essential to watch all individuals in each pen, and since there was only one

observer, this meant that time spent observing each bird was limited. Care was taken to ensure that the observations were as representative as possible despite small behavioural samples for each individual.

2.5 Pecking damage scores

Birds were inspected for pecking damage at the times 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 as follows: 0, no damage; 1, slight feather damage/loss with no bare skin; 2, damage with $<1\text{cm}^2$ bare skin; 3, moderate damage with up to $5 \times 5\text{cm}$ bare skin, or $<1\text{cm}^2$ bare skin with minor haemorrhage; 4, significant damage with $>5 \times 5\text{cm}$ bare skin, or up to $5 \times 5\text{cm}$ bare skin with minor haemorrhage; 5, significant ($1\text{-}2\text{cm}^2$) haemorrhage, or $>5 \times 5\text{cm}$ bare skin with minor ($<1\text{cm}^2$) haemorrhage. Each body area was scored separately and the values were summed to give a total for each bird.

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 as a rule, and more frequently if an outbreak of damaging pecking was taking place. Any bird found with a wound exceeding 2cm^2 in area was culled as soon as possible by intravenous injection of 2ml pentobarbitone sodium (Euthatal, Rhône Mérieux).

After discussion with the Roslin Institute Ethical Committee and the Roslin Institute veterinary surgeon a rule was agreed that the level of mortality due to injurious pecking at which a group of birds should be removed from an experiment should be 33% (four out of twelve birds). This rule had to be applied in two experiments (Chapter 3 and Chapter 5). All feather pecking work was done by authority of UK Home Office Project and Personal licences.

2.6 Egg production

The nature of the experimental set up in pens precluded records of egg laying being kept for individuals. Therefore, egg production was monitored on a per pen basis, with eggs collected daily and marked with the date and pen number. Eggs were stored in pen marked trays before being counted and bulk weighed at the end of each

week to provide pen means for egg number and egg weight. Abnormal eggs were identified, separated, and weighed individually. Egg abnormalities noted were suspected multiple yolks (large eggs), very small (less than 50% 'normal' size), lack of pigmentation (white) and 'waisted' (indicating two yolks). Soft-shelled eggs could not be monitored since birds usually ate these soon after they were laid.

2.7 Blood samples

To obtain a blood sample, birds were restrained on their sides with the brachial (wing) vein exposed. A 2ml blood sample was withdrawn by superficial venepuncture into a heparinised syringe (rinsed with 0.9% saline containing 0.28mg/ml Lithium-heparin salt). Temporary pressure was applied to the vein after removal of the needle to prevent haematoma. Blood was transferred immediately to 5ml blood collection tubes containing Lithium-heparin anti-coagulant (50 units) and mixed either by hand or by placing on rollers. Centrifugation at 1500 g (MSE-Mistral 2000R) for 7 minutes allowed separation of plasma which was split into 2-4 aliquots and immediately frozen and stored at -20°C until analysis.

For practical reasons, it was not possible to blood sample all birds involved in the large behavioural development experiments, and only a subset of birds in each group were sampled in these studies. In every experiment, blood samples were obtained from birds in the afternoon, to avoid potential interference from pre-ovulatory hormone surges, and pens and individuals were sampled in random order.

2.8 Assays

Unless otherwise described, assays were carried out on whole plasma. Where appropriate, concentrations of plasma steroid (oestradiol, progesterone and testosterone) were determined by radioimmunoassay. Given the high cost of these assays, measurements of plasma zinc and triglyceride (by colourimetric assay) were also employed to assess reproductive state. Plasma zinc accurately reflects levels of the lipophosphoprotein yolk precursor vitellogenin, plasma concentrations of which increase prior to the first oviposition and are maintained thereafter (Mitchell and Carlisle, 1991). Plasma triglyceride is also an indirect index of reproductive state, as it is a measure of circulating concentrations of triglyceride-rich very low density

lipoproteins (VLDL's) which are synthesised in the liver in response to oestradiol stimulation (Hermier et al., 1996).

2.8.1 *Triglyceride*

Plasma triglyceride concentrations were determined using a commercially available diagnostic kit (Triglyceride L-type, Wako Chemicals) modified for use with 96 well microplates and an automatic microplate reader (Dynatech MR5000). This end point colourimetric assay uses tri-olein as standard. The reaction of two colour reagents with triglyceride produces a blue pigment, the intensity of which is proportional to the triglyceride concentration in the sample. 6 μ l of standards (0.75, 1.50 and 3.00 mg/ml), blank (distilled water) and plasma samples were pipetted in duplicate into individual microplate wells. The limit of linearity for the assay is 20 mg/ml, so highly lipaemic plasma was diluted 5-fold with distilled water. Colour reagent A (200 μ l) was added to all wells, followed by colour reagent B (100 μ l) five minutes later. Plates were read at 595 nm after 10 minutes at room temperature and mean optical densities of duplicate samples were used to calculate plasma triglyceride concentration.

2.8.2 *Zinc*

Plasma zinc was also measured in microplates using an adapted commercially available diagnostic kit (Zn, Wako Chemicals). Precipitation of proteins leaves zinc in solution to bind to 5-Br-PAPS forming a reddish-violet chelate, which is measured by this end-point colourimetric assay. Plasma samples (100 μ l) were deproteinised by addition of an equal volume of 7% trichloroacetic acid and centrifugation at 2500 g for 10 min. 40 μ l of standards (0.5, 1.0 and 2.0 μ g/ml), blank (distilled water) and sample supernatant were pipetted in duplicate into individual microplate wells. Assay reagent was prepared immediately prior to use (by mixing colour reagents A and B in a 4:1 ratio). 200 μ l of reagent was added to each well, and plates were read at 560 nm after 10 minutes at room temperature. Mean optical densities of duplicate samples were used to calculate plasma zinc concentration.

2.8.3 *Gonadal steroids*

Plasma oestradiol was measured using a commercially available double antibody radioimmunoassay kit with a modified extraction. Full details of the procedure and its validation are given in Appendix B.

Plasma progesterone and testosterone concentrations were measured using commercially available coated-tube radioimmunoassay kits (Coat-a-count Total Testosterone and Coat-a-count Progesterone, Diagnostic Products Corporation). Both kits had similar protocols, involving addition of standards (provided with the kits) and samples (whole plasma) to antibody coated tubes, addition of radioactive tracer (iodinated progesterone or testosterone) followed by an incubation (3 hours at room temperature for progesterone, 3 hours at 37°C for testosterone). After aspiration of liquid from the tubes, their ¹²⁵I content was determined using a gamma counter (1277 Gammamaster, Wallac). Appropriate calculations to determine hormone concentration in samples from standards were carried out on a spreadsheet (Excel 97).

2.9 **Hormone implants**

Three implants were used in the experiments described in this thesis, one commercially available pellet implant and two custom-made Silastic implants. These are illustrated in Plate 2.1.

2.9.1 *Slow release pellets*

Pellet hormone implants with 60-day slow release (Innovative Research of America, Sarasota, Florida, USA) were used in Chapter 4, Experiments 1a and 1b (Plate 2.1 c). These implants are used primarily in small mammals and are designed to provide constant release of the desired substance from a biodegradable matrix. Combination pellets (6mm diameter) containing 10mg oestradiol and 25 mg progesterone were used, to deliver 0.167 mg/day oestradiol and 0.417 mg/day progesterone for 60 days. The pellets were implanted subcutaneously in the neck under either local or general anaesthesia (see Section 2.10).

2.9.2 *Silastic implants*

Silastic implants are lengths of flexible silicone tubing filled with crystalline hormone or drug. The semi-permeable tubing allows body fluids to enter the implant and its contents are released. The rate of delivery depends on solubility of contents, tubing wall thickness and surface area. Construction of Silastic implants took place in a fume cupboard with appropriate safety precautions for working with pure crystalline hormones (protective clothing and face mask). Medical grade translucent Silastic tubing (Dow Silastic[®], Sani-tech (UK) Ltd, Hampshire, UK) was cut into lengths 1cm longer than the required implant length. Silicone sealant (Dow Corning[®]) was squeezed from a 1ml syringe into one end of the tubing to fill the first 5mm of its length creating a seal. Once the sealant was dry, a small spatula was used to fill the implant with crystalline hormone via the open end. The implant contents were packed down periodically by inserting a length of cannula tubing (1mm diameter) into the lumen of the tubing. When the implant was full to within 5mm of its length, the open end was filled with sealant. Implants were wiped with cotton wool soaked in 70% ethanol then primed prior to implantation by soaking in 0.9% saline overnight. Two different Silastic implants were constructed using tubing of differing lumen diameter and wall thickness (Plate 2.1 a and b); details are given in Chapter 4, Experiments 3 and 4. All Silastic implants were inserted subcutaneously in the neck under general anaesthesia (see Section 2.10).

2.10 **Surgical implantation**

2.10.1 *Local anaesthesia*

Birds were restrained on their sides and the feathers on the right side of the neck were parted to expose the skin. Any small downy feathers present on the skin surface were removed and the area was wiped clean with 70% ethanol. 1-2 ml local anaesthetic (Xylocaine, Astra Pharmaceuticals) was injected subcutaneously over a 3cm² area. The skin was pinched and raised and a 5-10 mm incision was made. A subcutaneous pocket was made by blunt dissection with minimal damage to the underlying tissue. The implant was inserted and the incision was closed with one or two separate sutures. The procedure was completed within 5 minutes and birds were returned to their home pen. The implantation site was invisible externally due to feather cover. Regular postoperative checks were carried out on implanted birds.

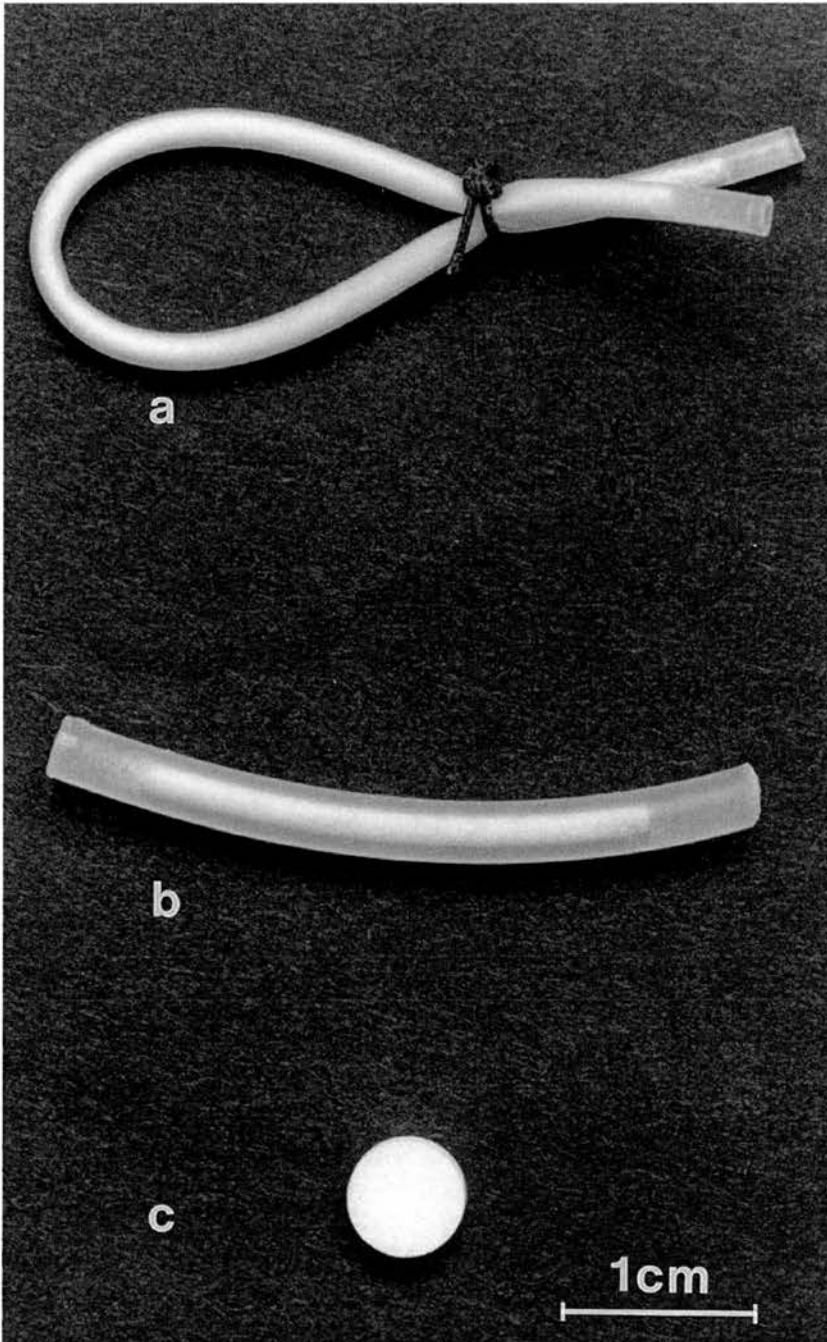


Plate 2.1 Types of hormone implant employed in this study. a. large Silastic implant; b. small Silastic implant; c. pellet implant.

2.10.2 *General anaesthesia*

Food was withdrawn approximately 12 hours before surgery. General anaesthesia was induced during light restraint by administration of 2% halothane (Fluothane, ICI Ltd UK) in pure oxygen at a rate of 1.6-1.8 l/min (depending on bird weight) through a rubber tube partially inserted into the buccal cavity and held in place with adhesive tape. A satisfactory plane of anaesthesia was confirmed by absence of withdrawal reflexes to comb pinches. Anaesthesia was maintained by delivery of 1-1.5% halothane in oxygen at 1.6-1.8 l/min. Birds were placed on their sides on a heated operating table and the feathers on the right side of the neck were parted to expose the skin. Any small downy feathers present on the skin surface were removed and the area was wiped clean with 70% ethanol. The skin was pinched and raised to make a 5-10 mm incision. A subcutaneous pocket (size in relation to implant size) was made by blunt dissection with minimal damage to the underlying tissue. The implant was inserted and the incision was closed with 2-4 separate sutures. If a bird was to receive two implants the procedure was repeated on the other side of the neck. As above, the implantation sites were invisible externally. The procedure was completed within 15 minutes and birds were revived by delivery of pure oxygen (flow rate 1.6-1.8 l/min). Birds were then placed alone in a crate in a quiet room and observed frequently during recovery. Regular postoperative checks were also carried out after birds were returned to their home pen.

2.11 **Statistical analysis**

Many of the data obtained in this study were repeated measures. This type of data presents problems with independence, since measurements made close together in time are likely to be more similar (i.e. less independent) than measurements made further apart in time. Various techniques were employed to analyse repeated measures data appropriately, such as split plot analysis of variance with ages as plots (Chapter 3), separate analyses at each time of measurement (Chapter 4), and analysis of data summarised into time blocks (Chapter 5). Many data obtained were not distributed normally. In these cases, non-parametric analyses (e.g. Spearman rank correlation) or transformation of the data (e.g. log transformation) were applied as appropriate. Details of the statistical approaches used are given in each chapter. All

statistical analyses were carried out using Genstat Version 4.1 or Minitab for Windows (Release 12).

Chapter 3

Behavioural and hormonal development of pullets

3.1 Introduction

Developmental aspects of feather pecking and cannibalism have been the subject of a growing number of investigations in recent years, with emphasis on external factors such as food form (Savory and Mann, 1997), light (Kjaer and Vestergaard, 1999), and floor type (Blokhus and Van der Haar, 1992; Norgaard-Nielsen et al., 1993; Huber-Eicher and Wechsler, 1997; Johnsen et al., 1998). Examination of the influence of such environmental factors during rearing can improve our understanding of the causation of feather pecking, and may also show how pecking problems in adulthood may be avoided by rearing in appropriate conditions. Floor substrate during rearing, in particular, has been found to have a profound effect on propensity to feather peck. Johnsen et al. (1998), for example, showed that chicks reared on sand and straw exhibited no feather pecking, while those reared on wire showed severe feather pecking and cannibalism.

Less is known, however, about intrinsic developmental changes in behaviour, which could account for age related peaks in feather pecking observed under constant environmental conditions. Several authors have reported a peak in pecking during the growing period between four and 10 weeks of age (Hughes and Duncan, 1972; Hughes 1973; Kjaer and Sorensen, 1997) and also that pecking problems tend to begin or worsen at sexual maturity (Dickerson and Kashyap, 1961; Hughes and Duncan, 1972; Allen and Perry, 1975; Blokhus, 1991; Norgaard-Nielsen et al., 1993). These distinct behavioural phases suggest that internal factors are potentially of great importance, particularly at sexual maturity, a time when profound physiological changes occur in preparation for the onset of lay. Involvement of gonadal hormones in the development of feather pecking was demonstrated by Hughes (1973), who showed that increased feather pecking damage normally seen at the onset of lay was advanced in juvenile birds treated with a combination of exogenous oestradiol and progesterone. This remains the only research linking feather pecking and hormonal state, but it was limited because only pecking damage

scores were measured and not plasma hormone levels or behaviour. A recent study (Savory and Mann, 1997) provided detailed information on age related behavioural changes in pullets, but did not relate these to hormonal state.

The current experiment aimed to provide detailed information about various aspects of pullet development, including behaviour, growth and hormonal state. The approach adopted was to examine long term behavioural development of pen-housed pullets, with particular attention to behavioural and hormonal changes at sexual maturity. Bird to bird pecking was examined in detail, recording numbers and types of pecks delivered and areas of the body targeted. All observations of pecking were carried out on an individual bird basis, to allow identification of peckers and peckees. Given the known unpredictability of feather pecking in commercial laying strains, there was no guarantee that pecking would occur, so the study was also designed to investigate the incidence of pecking damage under a given set of environmental conditions. As such, birds were housed under conditions known to increase the likelihood of damaging pecking (high stocking density, relatively bright lighting and pelleted food). The experiment also aimed to verify the accuracy of the indirect indices of reproductive state, triglyceride and zinc (Mitchell and Carlisle, 1991), as a basis for further studies.

3.2 Methods

One hundred and forty four ISA Brown layer pullets were used in this experiment. They were randomly allocated at day old into 12 groups of 12 and were housed and reared in pens to 26 weeks of age as described in Section 2.2. Regular measurements of growth, behaviour (ethogram behaviours and pecking counts, see Section 2.4), pecking damage scores, egg production and blood sampling were carried out as described in Chapter 2, according to the schedule shown in Table 3.1. Collection of blood samples and measurement of comb and wattle growth were carried out on a subset of 3 birds (leg band colours blue, red, and green, assigned at random) in each pen in alternate weeks from 10 weeks of age. Blood samples were assayed for plasma zinc and triglyceride (See Section 2.8), and a limited number of samples were assayed for oestradiol, progesterone and testosterone as described in Chapter 2.

<i>Week</i>	1	2	3	4	5	6	7	8	9	10	11	12	13
Body weight		✓		✓		✓		✓		✓		✓	
Comb and wattle growth*										✓		✓	
Ethogram		✓		✓		✓		✓		✓		✓	
Bird to bird pecking					✓	✓	✓	✓	✓	✓	✓	✓	✓
Pecking damage scores		✓		✓		✓		✓		✓		✓	
Egg Production													
Blood Sampling*										✓		✓	

<i>Week</i>	14	15	16	17	18	19	20	21	22	23	24	25	26
Body weight	✓		✓		✓		✓		✓		✓		✓
Comb and wattle growth*	✓		✓		✓		✓		✓		✓		✓
Ethogram	✓		✓		✓		✓		✓		✓		✓
Bird to bird pecking	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Pecking damage scores	✓		✓		✓		✓		✓		✓		✓
Egg Production			✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Blood Sampling*	✓		✓		✓		✓		✓		✓		✓

Table 3.1 Schedule of measurements in the behavioural development experiment, those marked with * were carried out on the same subset of 3 birds per group.

3.3 Results

3.3.1 *Body weight*

A smooth growth curve was observed (Figure 3.1), which began to level off after sexual maturity (16-20 weeks of age), when the birds weighed on average 1.6 kg, rising to about 1.8 kg at the end of the experiment (26 weeks). Variation between birds was small reflecting fairly uniform weight gain, with a mean coefficient of variation across ages of 0.022.

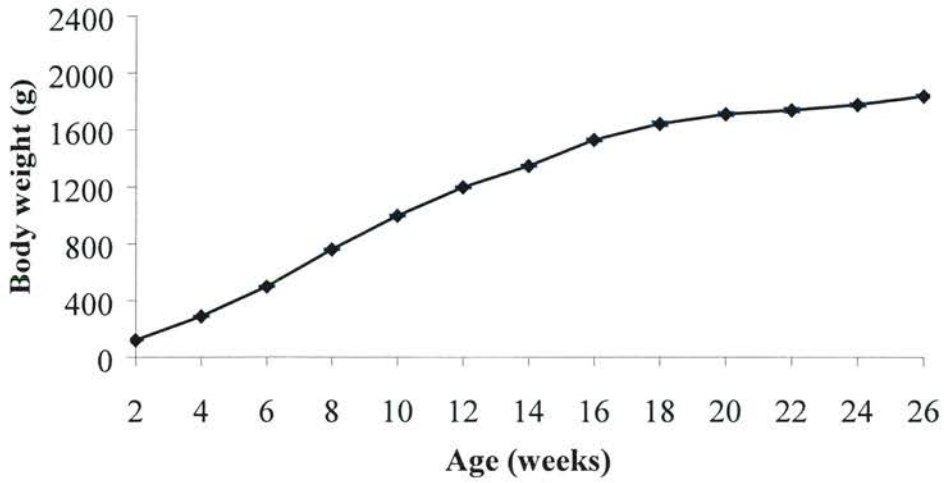


Figure 3.1 Mean body weights (\pm SE) of 12 groups of pullets in 12 pens from 2 to 26 weeks (n=144)

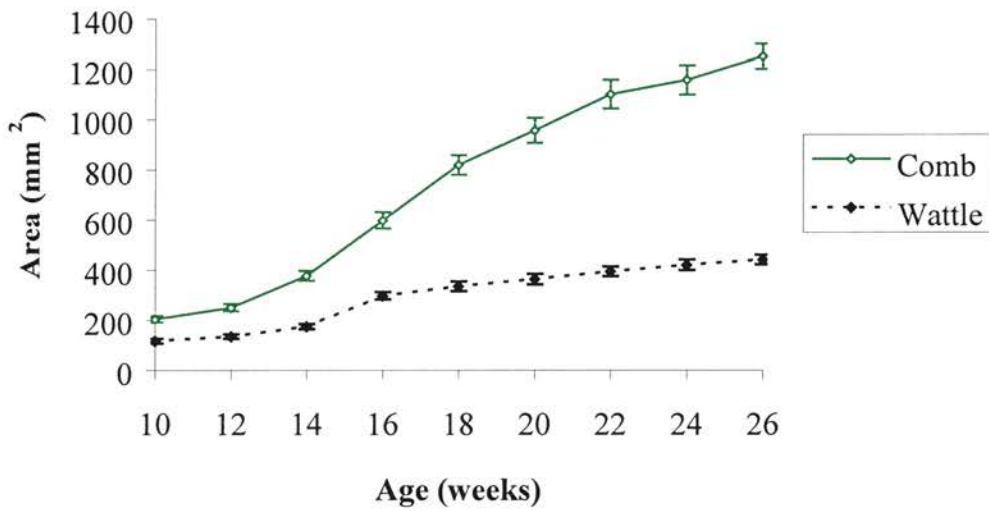


Figure 3.2 Mean comb and wattle growth (\pm SE) in pullets from 10 to 26 weeks (n=36)

3.3.2 *Comb and wattle growth*

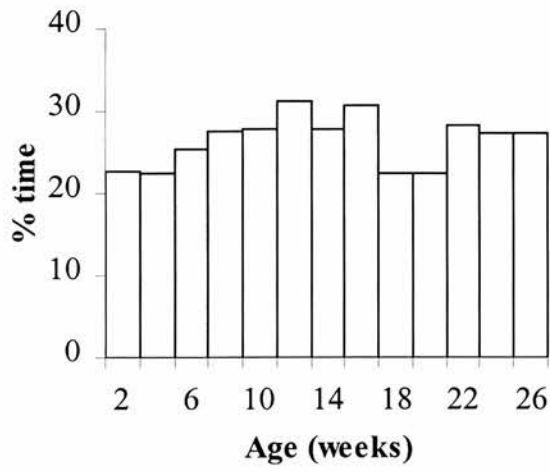
Age related changes in comb and wattle growth are shown in Figure 3.2. Comb and wattle size increased most rapidly after 14 weeks; combs grew faster than wattles and were still growing at the end of the experiment. Comb size was less variable (maximum CV= 0.21) than wattle size (maximum CV= 0.41).

3.3.3 *Ethogram*

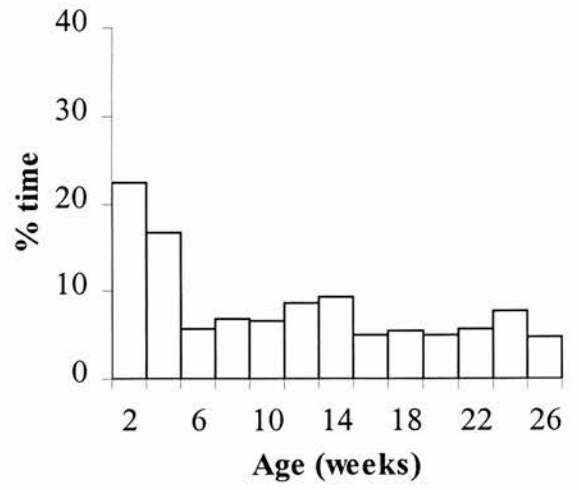
Birds spent most time standing, walking and in litter directed behaviour (26, 25 and 17% of time across all ages), and less time sitting, preening and feeding (9, 11 and 8% of time across all ages) (Figure 3.3). Drinking, dustbathing and pecking other birds each took up less than 5% of the time budget across all ages. Egg production had commenced in all groups by 16 weeks (see Section 3.3.6) and this was chosen as the age of sexual maturity to make comparisons of ethogram behaviours before (4-14 weeks) and after (16-26 weeks) the onset of lay. Significant paired t-test comparisons (Table 3.2) showed that time spent preening and dustbathing increased after the onset of lay, while time spent sitting decreased.

3.3.4 *Bird to bird pecking*

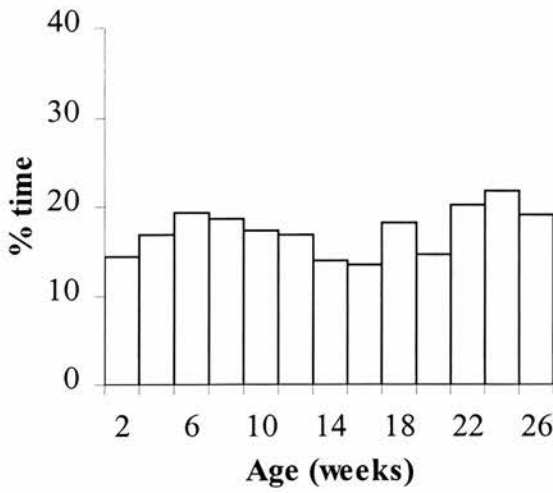
Age related changes were observed in counts of non-aggressive pecks (Figure 3.4.1), feather pulls (Figure 3.4.2), aggressive pecks (Figure 3.4.3) and feather eating events (Figure 3.4.4). Non-aggressive pecks were the commonest type of pecks seen accounting for 89.0% of all pecks/pulls observed. Numbers of non-aggressive pecks observed varied widely between pens and with age, showing an increase at the onset of sexual maturity (between 16-18 weeks). Although feather pulls and aggressive pecks were rarer, accounting for 5.2% and 5.8% of pecks/pulls, respectively, much clearer increases in these behaviours were seen at the onset of lay. More feather eating events were seen in the growing period (6-12 weeks) than after the onset of lay. Applying the same before and after onset of lay comparisons to the pecking counts (Table 3.2) confirmed that non-aggressive pecks, aggressive pecks and feather pulls increased, while feather eating events decreased after sexual maturity.



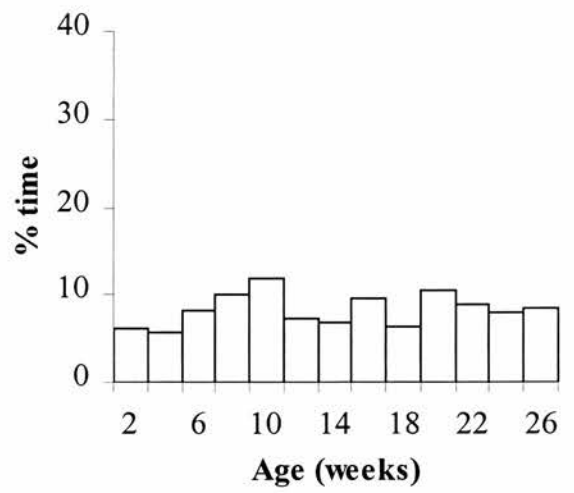
3.3.1 Standing



3.3.2 Sitting

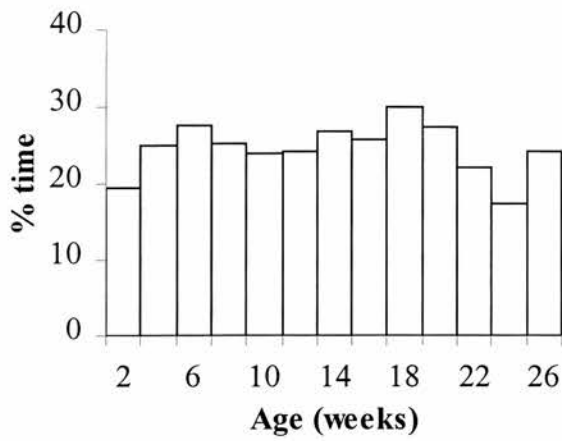


3.3.3 Walking

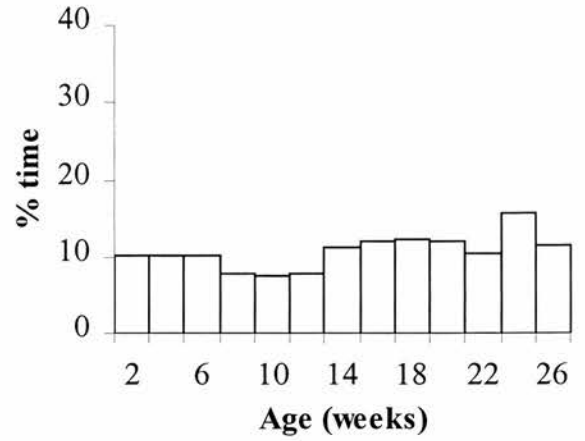


3.3.4 Feeding

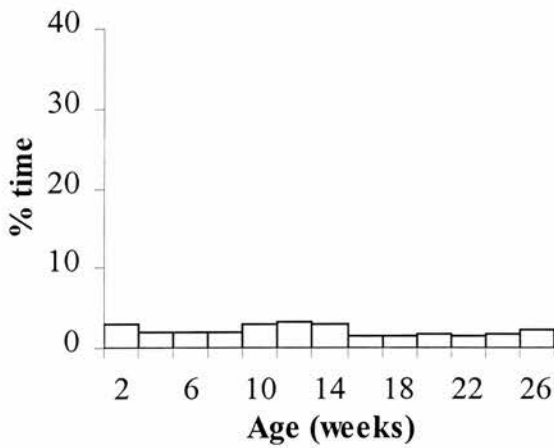
Figure 3.3 Mean % time spent in ethogram behaviours across all pens between 2 and 26 weeks.



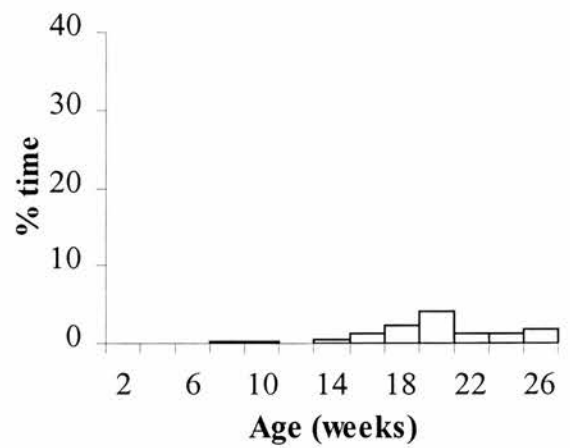
3.3.5 Litter directed behaviour



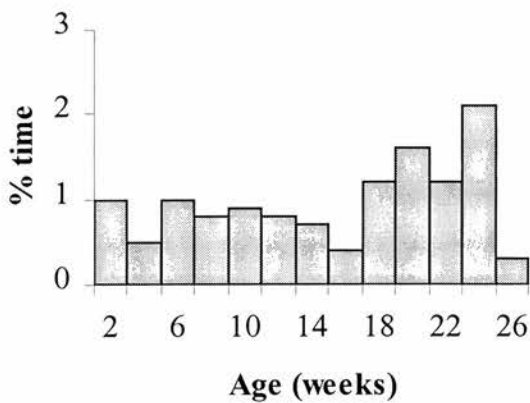
3.3.6 Preening



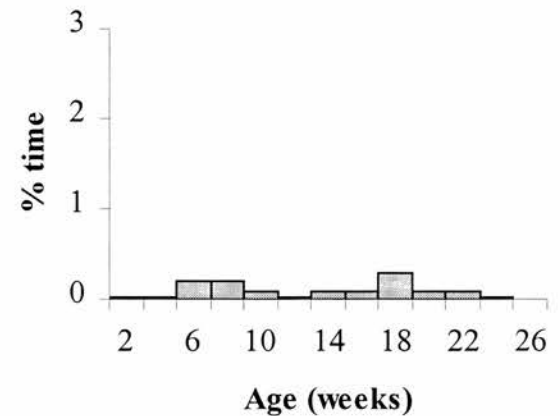
3.3.7 Drinking



3.3.8 Dustbathing



3.3.9 Non-aggressive pecking



3.3.10 Aggressive pecking

Figure 3.3 Mean % time spent in ethogram behaviours across all pens between 2 and 26 weeks. Note the reduced y-axis scale on Figures 3.3.9 and 3.3.10.

	Before sexual maturity	After sexual maturity	Paired t test
Ethogram (mean % time± SE)			
Standing	27.1 ± 0.7	26.7 ± 0.8	NS
Sitting	9.0 ± 1.0	5.4 ± 0.6	P < 0.01
Walking	17.2 ± 0.5	17.2 ± 0.6	NS
Litter Directed	25.5 ± 1.0	23.5 ± 1.0	NS
Feeding	8.4 ± 0.7	9.1 ± 0.6	NS
Drinking	2.5 ± 0.2	1.9 ± 0.2	NS
Preening	9.2 ± 0.6	12.6 ± 0.9	P < 0.01
Dustbathing	0.2 ± 0.1	2.2 ± 0.4	P < 0.001
Non-aggressive pecking	0.1 ± 0.0	0.1 ± 0.2	NS
Aggressive pecking	0.8 ± 0.1	0.9 ± 0.2	NS
Pecking counts (mean pecks/bird/2 min ± SE)			
Non-aggressive pecks	0.96 ± 0.10	1.37 ± 0.16	P < 0.05
Aggressive pecks	0.04 ± 0.01	0.12 ± 0.03	P < 0.001
Feather pulls	0.02 ± 0.01	0.13 ± 0.03	P < 0.01
Feather eating	0.06 ± 0.01	0.03 ± 0.01	P < 0.01

Table 3.2 Results of paired t tests comparing mean proportions of time spent in different behaviours and mean pecking counts, before and after sexual maturity.

Non-aggressive pecks were targeted at seven body areas, the back, beak, wings, tail, neck and head (Figure 3.5). Pecks at the back and beak were most common, accounting for 39% and 26% respectively. There were age related changes in the targeting of non-aggressive pecks, with pecks being aimed at the tail in the growing period (5-14 weeks), but at the back after sexual maturity. More detailed information concerning age related changes in targeting of non-aggressive pecks is given in Appendix C.

Within pens, individual variation in giving and receiving non-aggressive (feather) pecks was examined. Numbers of birds giving/receiving pecks at a range of rates was calculated (Figure 3.6). The pattern observed is similar to a negative exponential frequency distribution, with few birds giving/receiving many pecks and vice versa. This suggests that a small number of birds accounted for the majority of observed pecks. The effect appeared to be stronger for giving pecks than receiving pecks, with more high givers than high receivers.

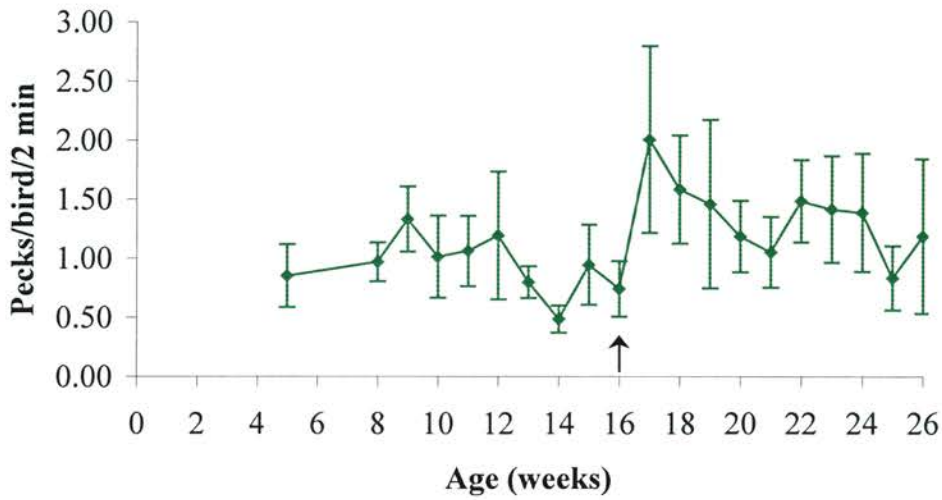


Figure 3.4.1 Non-aggressive pecks (↑ indicates the onset of lay)

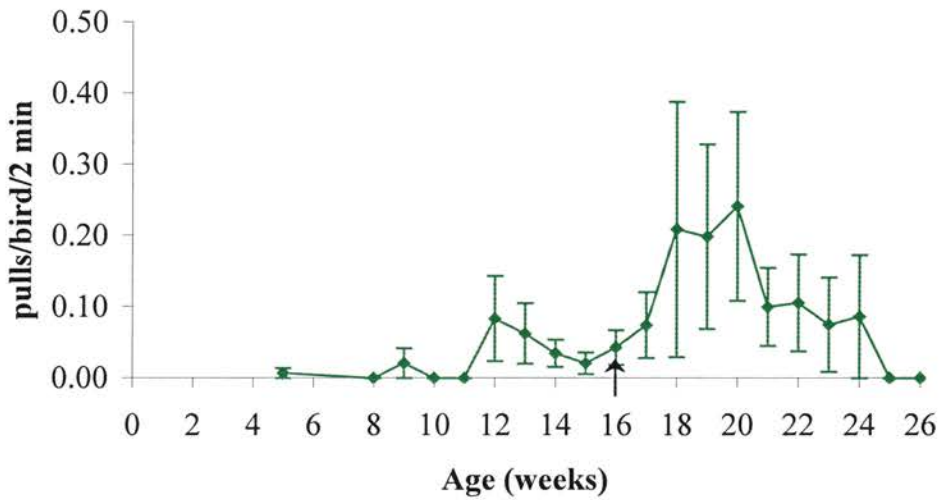


Figure 3.4.2 Feather pulls (↑ indicates the onset of lay)

Figure 3.4 Age related changes in mean numbers of observed non-aggressive pecks, feather pulls, aggressive pecks and feather eating events given and received per bird (\pm SE) across all pens from 5-26 weeks. Note the differing scales on each figure.

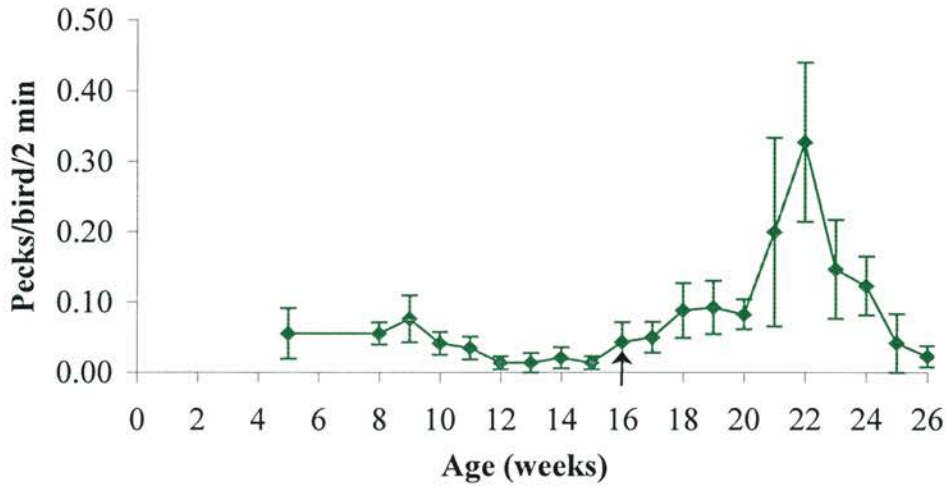


Figure 3.4.3 Aggressive pecks (\uparrow indicates the onset of lay)

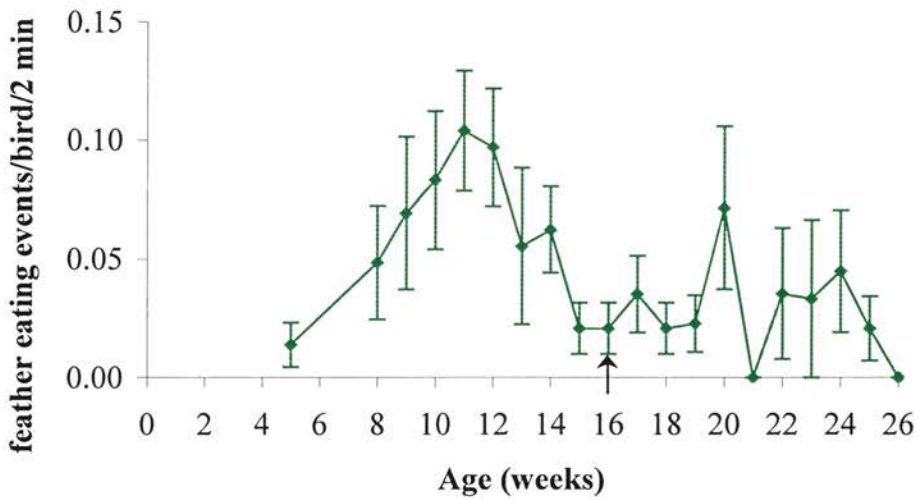


Figure 3.4.4 Feather eating events (\uparrow indicates the onset of lay)

Figure 3.4 Age related changes in mean numbers of observed non-aggressive pecks, feather pulls, aggressive pecks and feather eating events given and received per bird (\pm SE) across all pens from 5-26 weeks. Note the differing scales on each figure.

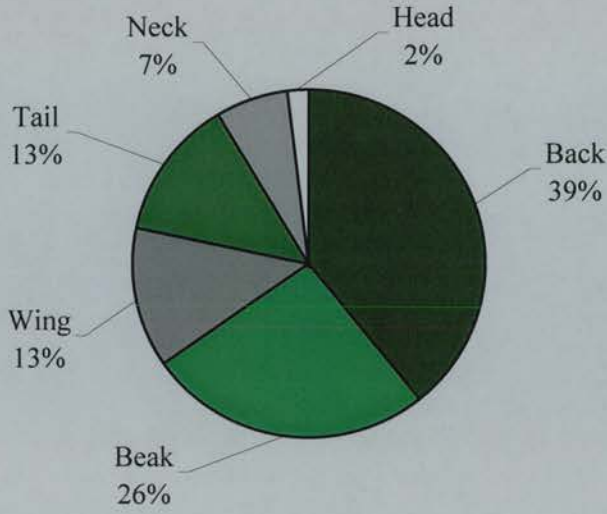


Figure 3.5 Overall mean percentages of non-aggressive pecks targeted at each body area.

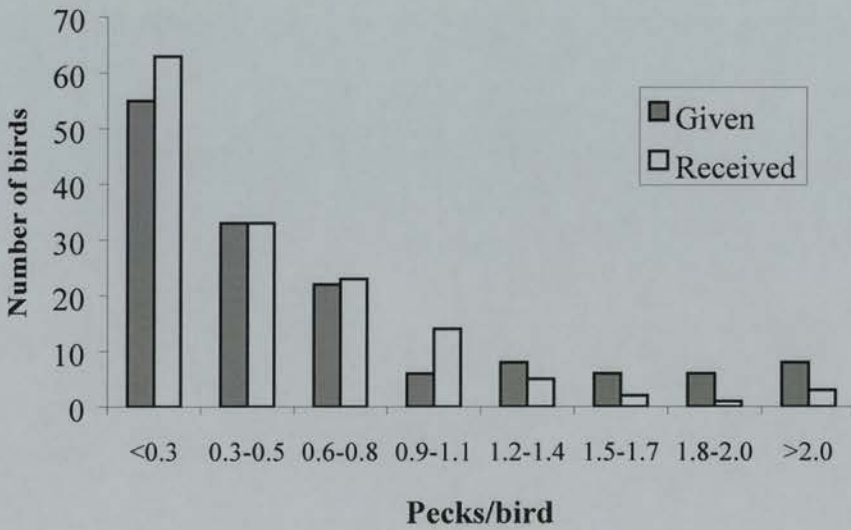


Figure 3.6 Frequency distribution of individual variation in rates of giving/receiving non-aggressive pecks

3.3.5 *Pecking damage scores*

Pecking damage was first seen at four weeks of age, and pecking damage scores were recorded from five weeks (Figure 3.7). The extent of pecking damage varied between pens, and was seen in the growing period only in Pens 1, 6, 7 and 10, and to a lesser extent in Pen 8. Between 4 and 8 weeks, pecks were directed particularly at the tail feathers, but some damage to back and wing feathers was also recorded. After eight weeks of age, little damage occurred and new feather growth resulted in a reduction in existing scores. Pecking damage began again at 16 weeks, when two birds in Pen 6 had to be culled due to destruction of the preen gland. An example of pecking damage to the back, tail, neck and wings is shown in Plate 3.1. The plot of mean pecking damage scores against age (Figure 3.7) indicates increasing pecking damage at sexual maturity, but does not reflect the situation in all pens. Injurious non-aggressive pecking occurred only in four pens, and this could be clearly seen from the pecking damage profiles of individual pens. Pens 1, 6, 7 and 10 exhibited steep increases in pecking damage at or after the onset of lay, and all had to be removed from the experiment prematurely, accounting for the reduction in pecking damage scores seen after 23 weeks (Figure 3.7, see Section 2.5 for removal criteria). The clear distinction between groups in the amount of pecking damage observed allowed categorisation as either 'high pecking' or 'low pecking'. Mean total damage scores in the 'high pecking' pens 1,6,7 and 10 were 5.92, 3.17, 6.25 and 3.92, respectively.

In pens 2, 7 and 10, between 19 and 22 weeks, sustained overt aggression was observed in which some birds were the recipients of repeated attacks. Victims typically received beak inflicted wounds to the comb, bruising to the face and head and neck feather loss (Plate 3.2). 'Bullied' birds showed avoidance and submissive behaviour and in some cases were prevented from feeding. Some birds were culled, according to the severity of aggressive pecking damage and the degree of weight loss. There were 23 deaths during the experiment, due to either feather pecking and cannibalism, aggression or other causes (Table 3.3), 10 of these were culls. Post mortems carried out on birds whose cause of death was not immediately apparent revealed causes such as egg peritonitis and ovarian tumour. Vent pecking accounted for 4 of the 14 deaths due to cannibalism, other sites of attack were the tail and the

base of the back, particularly the preen gland area. Overall, areas of the body contributing to damage scores were the back (34%), tail (30%), wings (7%) with the remainder being neck, head and comb scores reflecting aggressive pecking.

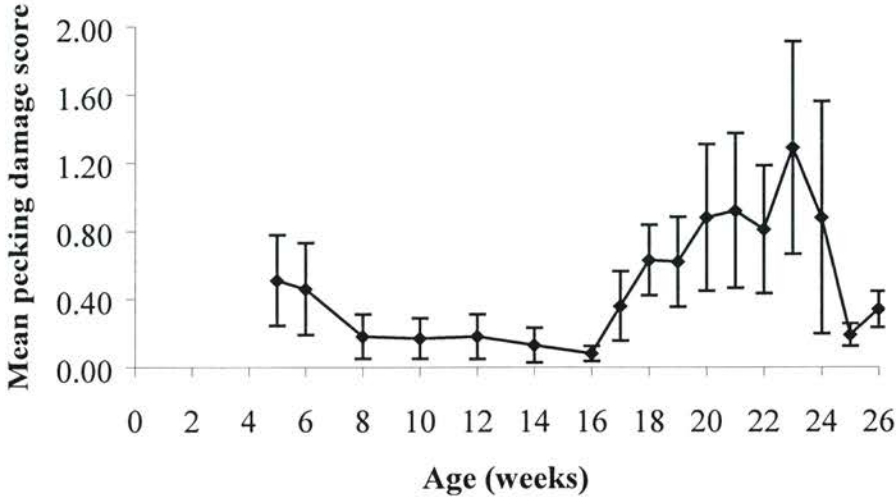


Figure 3.7 Mean pecking damage scores across all pens (± SE) from 5 to 26 weeks of age.

	Age (weeks)										
	16	17	18	19	20	21	22	23	24	25	26
Pen 1				●●	●	●●	—	—	—	—	—
Pen 2				○	○						
Pen 3											
Pen 4											
Pen 5											●
Pen 6	●●				●				●	—	—
Pen 7						●	○	●	●	—	—
Pen 8		●									
Pen 9		●									
Pen 10							○	●●	—	—	—
Pen 11							○				
Pen 12											●

● Cannibalism ○ Aggression ● Other causes
 — group removed from experiment (see Section 2.5)

Table 3.3 Number and types of mortality per pen between 16 and 26 weeks. Each dot represents one bird.



Plate 3.1 An example of pecking damage to the neck, back, tail and wings in a 49 week old ISA Brown hen.



Plate 3.2 Pecking damage to the comb, head and neck and bruising of the face as a result of aggressive pecking in a 23 week old ISA Brown hen.

3.3.6 Egg production

The first egg was laid at 15 weeks of age, but egg laying in all pens did not begin until 16 weeks. Egg production increased rapidly to 0.60 eggs/hen d at 18 weeks, and more gradually to about 0.70 eggs/hen d thereafter (Figure 3.8). Numbers of abnormal eggs fell with age (Figure 3.8), representing 20.1% of production at 16 weeks but only 2.5% at 26 weeks. The most common egg abnormality was double/multiple yolks (93%), followed by misshapen eggs (3%), small eggs (2%), soft-shelled and unpigmented eggs (both 1%). Mean (normal) egg weight increased steadily with age, from $41.1 \pm 3.21\text{g}$ at 16 weeks to $58.5 \pm 1.07\text{g}$ at 26 weeks. Egg eating was seen during behaviour observations in six pens (Pens 4, 5, 6, 8, 10 and 11).

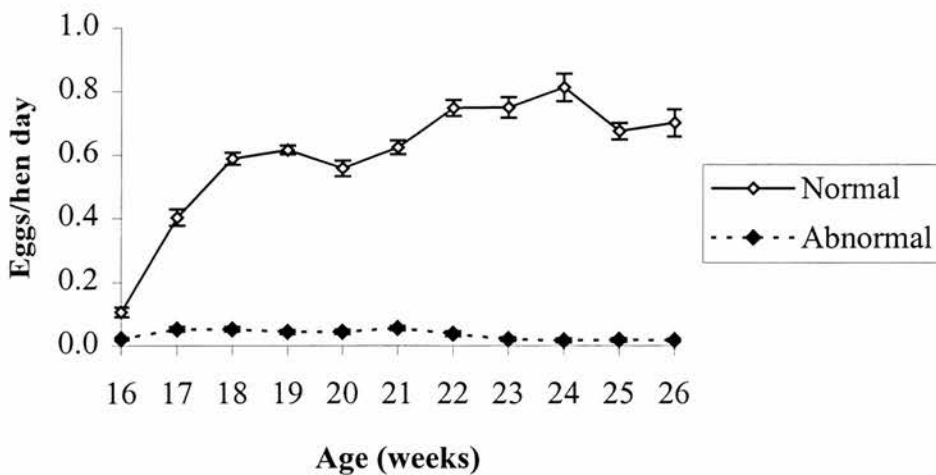


Figure 3.8 Mean production of normal and abnormal eggs/hen day (\pm SE) between 16 and 26 weeks of age.

3.3.7 Plasma assays

Age related plasma zinc and triglyceride profiles were similar. Both showed a large increase in concentration between 14 and 20 weeks of age (reflecting the onset of lay) and a smaller increase thereafter (Figure 3.9).

Plasma oestradiol concentration increased from approximately 100pg/ml at 12 weeks to a peak of 200pg/ml at 16 weeks, and then declined gradually (Figure 3.10). Plasma progesterone level increased between 12 and 20 weeks, from less than 0.4ng/ml to 1.2ng/ml, and then declined slightly. Plasma testosterone concentrations tended to decrease with age.

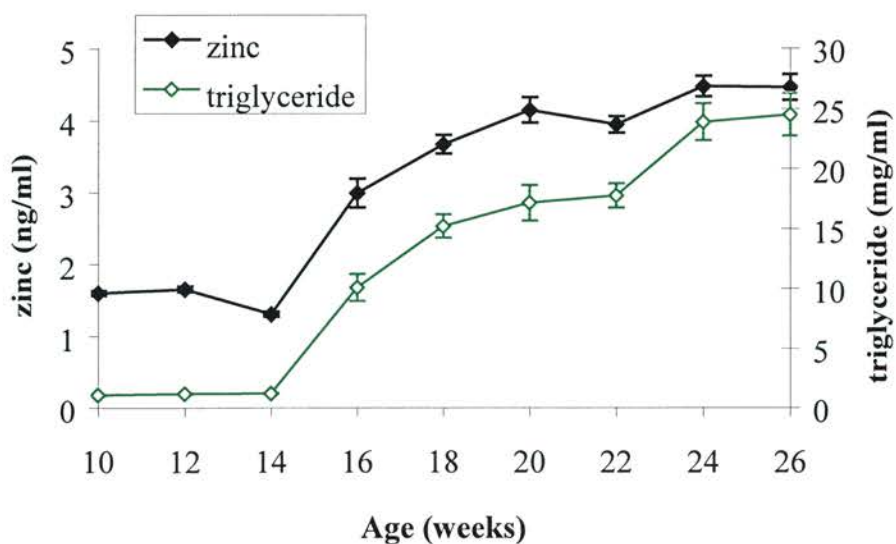


Figure 3.9 Age related changes in mean plasma zinc and triglyceride concentrations (\pm SE) between 10 and 26 weeks (n=36 at each age).

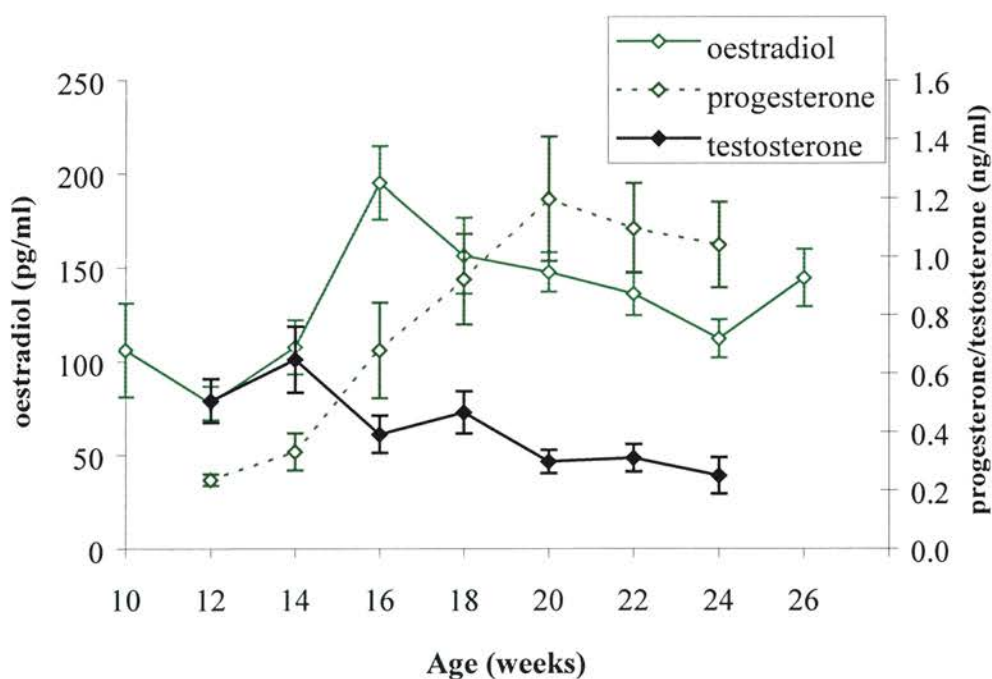


Figure 3.10 Age related changes in mean (\pm SE) plasma oestradiol (n=24 at each age) progesterone (n=12 at each age) and testosterone (n=12 at each age) concentrations.

3.3.8 Comparisons between 'high' and 'low' pecking pens

'High' pecking pens were defined as pens in which deaths had occurred as a result of damaging pecking. 'Low' pecking pens were those in which little or no pecking damage has occurred. Split plot ANOVAs were carried out on ethogram behaviours, pecking counts and egg production data to determine effects of age, 'high' or 'low' pecking and the interaction between them (Table 3.4). Significant effects of age alone were observed for proportions of time spent sitting, walking and preening. 'High' and 'low' pecking groups differed significantly in the time spent in non-aggressive pecking, and in numbers of aggressive and non-aggressive pecks observed. Numbers of feather pulls and feather eating events recorded were greater in high pecking groups. There were significant interactions between age and high or low pecking, with time spent in non-aggressive pecking and counts of feather pulls. In high pecking groups, both these measures increased markedly after the onset of lay, while in low pecking groups they remained consistently low. There was no difference in egg production between high and low pecking groups.

Ethogram	Age	High vs Low	Interaction
Standing	NS	NS	NS
Sitting	P < 0.05	NS	NS
Walking	P < 0.05	NS	NS
Litter Directed	NS	NS	NS
Feeding	NS	NS	NS
Drinking	NS	NS	NS
Preening	P < 0.05	NS	NS
Dustbathing	NS	NS	NS
Non-aggressive pecking	NS	P < 0.05	NS
Aggressive pecking	NS	P < 0.01	P < 0.05
Pecking counts			
Non-aggressive pecks	NS	P < 0.05	NS
Aggressive pecks	NS	NS	NS
Feather pulls	NS	P < 0.01	P < 0.05
Feather eating	NS	P < 0.05	NS
Egg production	P < 0.01	NS	NS

Table 3.4 Results of split-plot ANOVA analysis examining the effects of age, 'high' and 'low' pecking categories and their interaction.

3.4 Discussion

The developmental data obtained in this study for body weight, comb and wattle growth and ethogram behaviours provide a baseline for future studies. Age related changes in standing, sitting, feeding, preening and dustbathing were comparable to those found by Savory and Mann (1997). The pullets in the present study spent more time walking, in litter directed behaviour and in bird to bird pecking (non-aggressive and aggressive) than reported previously (Savory and Mann, 1997). Pecking damage, where it occurred, was first seen in juveniles between 4 and 8 weeks, but was not severe enough to result in mortality until after the onset of lay. Thus, the results support the notion that damaging pecking increases at sexual maturity. Interestingly, preening and dustbathing, both feather-related behaviours, increased significantly after sexual maturity.

Variation among groups in the amount of pecking damage observed provided an opportunity to compare the behaviour and egg production of 'high' and 'low' pecking groups. However, of all ethogram behaviours, only bird to bird pecking differed between 'high' and 'low' pecking groups. Previous workers have attempted to identify behavioural correlates of feather pecking, with limited success. Keeling and Jensen (1995) found that cannibalistic hens were more active than non-cannibalistic hens, but found no differences in comfort, aggressive or social behaviours. Savory and Griffiths (1997) found no association between feather pecking and pulling and other oral behaviours, except that high receivers of feather pulls preened more. Within 'high' pecking groups, only a few individuals were responsible for the damaging pecking, and it is conceivable that the ethogram of these individuals was different in some respects, but was diluted by the behaviour of low pecking pen-mates.

Results from detailed observations of bird to bird pecking also showed that non-aggressive pecks, feather pulls and aggressive pecks increased after the onset of lay. Feather eating events decreased after the onset of lay, after a peak in the growing period around 12 weeks of age (see Chapter 6 for a full examination of feather eating). Non-aggressive pecks were the most common type observed, as found by previous workers (Leonard et al. 1995; Savory and Griffiths, 1997). Feather pulls and aggressive pecks showed clear increases after the onset of lay, but

this increase was not so apparent for non-aggressive pecks, probably because the category included both gentle and vigorous pecks. Given the increase in pecking damage scores after the onset of lay, it is likely that if types of non-aggressive pecks had been recorded separately, an increase in vigorous pecks would have been observed. The reduction observed in pecking/pulling toward the end of the experiment is artificial, and was due to the removal of groups where severe pecking damage occurred.

When individual rates of giving and receiving non-aggressive pecks were examined, the frequency distribution was in the form of a negative exponential, with many more low givers/receivers of pecks than high givers/receivers. Similar distributions have been found elsewhere, with authors reporting 16% (Savory and Griffiths, 1997) and 12% (Wechsler et al., 1998) of the population as high givers of feather pecks. In this study, 5% of individuals were high peckers, defined as those in the highest 'bin' of pecking rate, with only 2% high receivers.

Overall, the majority of non-aggressive pecks were aimed at the back (39%), beak (26%), wings (13%) and tail (13%), a similar pattern to that seen in previous studies (Allen and Perry, 1975; Savory and Mann, 1997). Targeting of the back, wings and tail was reflected in pecking damage scores, which represented 34, 30 and 7% of total scores, respectively. The targeting of pecks showed age related changes, with the tail being targeted more in the growing period (between 5 and 14 weeks), and the back being targeted after the onset of lay (14 weeks onwards).

Outbreaks of severe aggression were seen in some pens after the onset of lay, with particular individuals being targeted. Attacks on 'runts' have been described in commercial flocks, birds whose consistent submissive behaviour appears to attract aggressive pecking (Wood-Gush, 1971; Appleby et al., 1992). The victims of aggressive attack in the present study did not appear to be runts from the start of experiment, and only lost weight as a result of bullying after the onset of lay. Aggressive pecking increased significantly after the onset of lay, but was not related to 'high' or 'low' feather pecking. The most severe aggression occurred in Pen 2, a 'low' feather pecking pen, and a death resulted from aggression in another 'low' group, in Pen 11. However, aggressive behaviour was also seen in two 'high' pecking groups, in pens 7 and 10. The severe aggression observed in some groups

appears to be a separate phenomenon to feather pecking and cannibalism, supporting previous studies finding no associations between aggressive pecking and feather pecking (Hughes and Duncan, 1972; Wood-Gush and Rowland, 1973; Leonard et al., 1995). It is unclear whether such aggressive behaviour could have similar injurious consequences in commercial flocks, since most production systems do not allow the formation of stable, small groups as were present in this experiment. Body weight has been (inconsistently) associated with dominance rank (Wood-Gush, 1971), although it is difficult to know whether birds are heavy because they are dominant, or vice versa. Body weights showed very low variability within and between groups, and it is possible that the lack of variation within groups could have contributed to increased levels of aggression.

Feather pecking and cannibalism accounted for 14 of the 23 deaths in this study. All the deaths/culls occurred after the onset of lay, in four groups that as a result were defined as 'high' pecking. In six cases the site of cannibalistic attack was the base and side of the tail, in three cases the preen gland was attacked and there were four mortalities as a result of vent pecking. Interestingly, the deaths due to destruction of the preen gland occurred just after the onset of lay, while vent pecking mortalities tended to occur slightly later. It has been suggested that oviposition may increase the attractiveness of the vent as a stimulus for pecking (Savory, 1995; Gunnarsson et al. 1999), it may be that more vent pecking took place late in the experiment when more birds were in lay.

Egg laying had commenced in all groups by 16 weeks of age, although not all birds in all pens would have been in lay until considerably later. The design of the experiment made it impossible to determine individual egg production and its relation to pecking behaviour. On a per pen basis, there was no evidence of a relationship between egg production and 'high' or 'low' pecking. Production in terms of egg number and egg weight was comparable to target production figures defined in the management guide for this strain, with over 80% production being reached by 26 weeks. More very large (probably multiple yolked) eggs were laid in the first six weeks of lay, and it is possible that oviposition of these could affect the appearance of the vent and its potency as a stimulus for pecking.

Mean plasma levels of oestradiol, progesterone and testosterone observed here, and the timing of changes, were similar to those reported in previous studies (Senior, 1974a; Williams & Sharp, 1977; Itoh et al., 1988). The rapid increases in plasma levels of zinc and triglyceride after 14 weeks of age confirm their validity as indirect indices of the onset of ovarian activity and steroid secretion and therefore reproductive state (Mitchell and Carlisle, 1991). The increased pecking/pulling in 'high' pecking groups coincided more closely with the rise in plasma progesterone concentration, and egg production, at the onset of lay than that of oestradiol which peaked before the onset of lay. This association does not, however, necessarily indicate cause and effect. The decision to blood sample the same subset of birds at each age (see Section 2.7) meant that the hormone results provided only general information about the timing of changes. Appreciable pecking damage occurred in only a third of pens, and in each pen only a quarter of birds was sampled. Furthermore, within 'high' pecking pens, only a small percentage of birds were responsible, which were not necessarily sampled, since the random selection of which birds to sample was made before any pecking damage occurred. This precluded comparison of hormonal state and behaviour within individual birds.

In conclusion, under constant environmental conditions, developmental changes were observed in the number, type and targeting of bird to bird pecks. Age related changes were also apparent in ethogram behaviours, with the feather-related behaviours, preening and dustbathing, increasing at sexual maturity. The variability between groups in the amount of pecking damage observed illustrates the unpredictability of the problem. These results support previous observations that pecking damage tends to worsen at the onset of lay and provide further circumstantial evidence for hormonal involvement in the aetiology of pecking damage.

Chapter 4

Linking behaviour and hormonal state

4.1 Introduction

Influences of gonadal hormones on the behaviour of female domestic fowl are poorly understood, and the few studies available have concentrated on behaviour associated with reproduction. Wood-Gush and Gilbert (1969) showed that chronic treatment (serial injections for up to three months) of immature hens with oestrogen induced nesting behaviour, consisting of pacing and a distinctive call. Wood-Gush and Gilbert (1975) reported that treatment with progesterone alone did not induce nesting behaviour in ovariectomised hens, but was highly effective in inducing nest entry and examination in hens previously treated with oestrogen. Declines in plasma oestradiol and progesterone concentrations are associated with broody behaviour (Bedrak et al., 1981; Kono et al., 1985), and increases in plasma oestradiol concentration have been observed when nesting is disrupted (Richard-Yris et al., 1998). An association between oestradiol and activity (increased exploratory behaviour in ovariectomised females after oestradiol administration) has been also demonstrated (Horne and Wood-Gush, 1970). Allee and Collias (1940) examined the effects of oestradiol on the social organisation in small flocks of hens. In staged contacts with unfamiliar birds, birds treated with oestradiol exhibited reduced aggressiveness and lost rank to birds lower in the pecking order.

Increases in damaging pecking at and after the onset of lay, which have been observed in several studies (Dickerson and Kashyap, 1961; Hughes and Duncan, 1972; Allen and Perry, 1975; Blokhuis, 1991; Norgaard-Nielsen et al., 1993; Bilcik and Keeling, 1999), have led to suggestions that feather pecking might have a hormonal basis. The existence of a relationship between pecking behaviour and hormonal state was also supported by the results presented in Chapter 3, where changes in pecking behaviour followed an increase in plasma oestradiol and coincided with the rise in plasma progesterone at the onset of lay. These temporal associations provide circumstantial evidence for a link, but the only evidence of a

causal relationship is from a study (Hughes, 1973), which implicated both oestradiol and progesterone in the development of pecking damage in pullets. In this study, Hughes (1973) demonstrated that the increase in pecking damage normally associated with the onset of lay could be reproduced in 12-week-old pullets treated chronically with a combination of oestradiol and progesterone implants. Treatment with progesterone alone caused a small but significant increase in damaging pecking, treatment with oestradiol had no effect, and treatment with testosterone had a suppressive effect on damaging pecking. This experiment, while valuable, was confined to examining pecking damage scores, and no attempt was made to measure plasma hormone levels or behaviour.

In the current studies, the overall aim was to repeat and extend the experiment carried out by Hughes (1973), by examining behavioural development in detail and measuring plasma hormone levels. Therefore, a hormonal manipulation was required that would mimic the hormonal state associated with sexual maturity for 4-6 weeks in 10-week-old commercial layer pullets. Various methods for administration of exogenous gonadal hormones are available, several of which have been applied to domestic fowl such as intravenous injection (Etches and Cunningham, 1976; Hagan et al., 1984), intramuscular injection (Wilson and Sharp, 1975; Wilson and Sharp, 1976) and subcutaneous implants (Johnson, 1983; Saito et al., 1986). In behavioural studies, care must be taken that the route of administration itself does not affect behaviour, and in the present study, implants seemed the best method for the chronic, easily applied manipulation required (relatively large numbers of birds were required because of the unpredictability of damaging pecking).

Whilst Hughes (1973) claimed successful manipulation of plasma steroids by implantation of crystalline pellets in immature pullets, a similar approach proved problematic in the present study. These difficulties and their causes were therefore addressed in an additional series of experiments which are described in this chapter.

Initial attempts to alter hormonal state using slow release pellet implants were unsuccessful (Experiment 1a and 1b), and were followed by trials investigating the effectiveness of a second implant type, constructed with Silastic tubing (Experiments 2a and 2b). In Experiment 3, a novel method of oral hormone administration was

evaluated. In the absence of an effective and reliable method of chronic oestradiol administration, Experiment 4 examined an alternative approach to modification of “oestrogen status” by means of acute administration of the anti-oestrogen, tamoxifen, and evaluation of the effects of this procedure upon pecking at a novel object by hens known to be ‘peckers’ or ‘non-peckers’.

4.2 Experiment 1a – Combination pellet implants

4.2.1 Introduction

This experiment aimed to examine in detail the behaviour (particularly bird to bird pecking) and hormonal state of pullets subjected to hormonal manipulation intended to mimic the onset of sexual maturity. As pullets approach sexual maturity, plasma oestradiol increases from c.100pg/ml to 300pg/ml three to four weeks before the first egg is laid (Senior, 1974a). Later, just before laying commences, plasma progesterone increases from under 300pg/ml to c.1000pg/ml (Williams and Sharp, 1977). Hence, both hormones show approximate three-fold increases (see Section 1.3). To manipulate hormonal state, Hughes (1973) used a combination of subcutaneous crystalline hormone pellets containing oestradiol (10mg) and progesterone (25mg). Such implants are no longer commercially available, and the closest available equivalent is slow release pellets (Innovative Research of America, Florida, USA) (Plate 2.1 c). These implants are designed to provide constant release of the desired substance from a biodegradable matrix and have been used successfully in rodents (e.g. Newbold et al., 1990). It has been reported that such pellet implants are effective for the administration of oestradiol to birds (Gordon et al., 1988) as assessed by changes in destabilisation of yolk precursor protein mRNAs in avian liver.

The approach used was a study of long-term behavioural development, similar to the experiment described in Chapter 3. Because of the unpredictability of pecking damage (damaging pecking was observed in only a third of groups in Chapter 3), only one hormone treatment was applied to the groups to maximise the likelihood of meaningful results. The treatment employed was a combination of oestrogen and progesterone (at the same dose found by Hughes (1973) to advance damaging pecking).

4.2.2 *Methods*

ISA Brown layer pullets were used in this experiment. 144 chicks were randomly allocated at day old into 12 groups of 12 and were housed and reared in pens to 26 weeks of age as described in Section 2.2. At day-old, groups were randomly assigned to 'control' or 'implant' treatments, six groups per treatment. At 10 weeks of age, every bird in the implant groups received a subcutaneous implant of one slow release hormone pellet (6mm diameter, containing 10mg 17 β -oestradiol and 25mg progesterone with a 60 day release period, see Section 2.9 for details) under the skin at the right side of the neck, using Xylocaine local anaesthetic (see Section 2.10). Control birds were sham implanted (identical surgical procedure but no pellet implanted). Birds and groups were treated in random order.

Regular measurements of growth (alternate weeks from 2 weeks of age), behaviour (pecking counts (non-aggressive pecks were categorised as gentle or vigorous) weekly from week 8 see Section 2.4), pecking damage scores (alternate weeks from 2 weeks), and egg production (weekly) were carried out as described in Chapter 2. All measurements at 10 weeks of age were carried out before implantation. Collection of blood samples and measurement of comb and wattle growth were carried out on a subset of 3 birds (leg band colours blue, red, and green) in each pen in alternate weeks from 10 weeks of age. Blood samples were assayed for plasma triglyceride, and a limited number of samples were assayed for oestradiol and progesterone (as described in Section 2.8).

Students t-test comparisons were carried out to determine treatment effects at each age for bodyweight, comb and wattle growth, pecking counts, pecking damage scores, egg production and plasma parameters.

4.2.3 *Results*

Bodyweights did not differ significantly between implant and control groups at any age, with normal growth observed in both treatments (data not shown). The oestradiol/progesterone implant treatment had a significant effect on comb and wattle growth (Figure 4.1), smaller combs being recorded in implant groups between 14 and 22 weeks of age ($P < 0.05$, t-test, $n = 12$) and smaller wattles in implant groups at 16 weeks ($P < 0.05$, t-test, $n = 12$).

The only effect of oestradiol/progesterone implant treatment on numbers of gentle pecks was at 16 weeks of age, when significantly more gentle pecks were observed in the implant groups (Figure 4.2). Significantly more vigorous pecks/pulls were observed in the implant groups at 12 weeks (Figure 4.3), and higher numbers of vigorous pecks/pulls were seen in control groups at 20 weeks. No significant differences were observed between treatments in numbers of aggressive pecks given or received (Figure 4.4).

No pecking damage was seen until 20 weeks of age, after which minimal damage scores (predominantly to the comb, head and neck) were recorded with mean scores less than one (data not shown). No pecking damage to the back, wings or tail was recorded and there were no deaths due to feather pecking and cannibalism during the experiment. Damage scores were not significantly affected by implant treatment at any age. Outbreaks of severe aggressive pecking in seven groups (Pens 1, 3, 7, 9, 10, 11, 12) resulted in 14 deaths, 11 of which were culls. The group in Pen 7 had to be removed from the experiment prematurely (at 22 weeks) as a result of aggressive pecking (see Section 2.5 for group removal criteria).

The first eggs were laid at 17 weeks, and all groups were in lay by 18 weeks. Egg production (Figure 4.5) was significantly reduced in implant groups between 19 and 21 weeks of age ($P < 0.05$, t-test, $n = 12$). Treatment had no significant effect on egg weight, which at 26 weeks was 58.7 ± 2.1 g in implant groups and 57.7 ± 0.21 g in control groups.

Plasma triglyceride profiles for implant and control groups were similar (Figure 4.6). Both exhibited a large increase between 16 and 22 weeks, reflecting the onset of ovarian activity, although triglyceride concentration was significantly lower in implant groups than control groups at 16, 18 and 24 weeks ($P < 0.05$, t-test, $n = 18$).

Implant treatment had no effect on plasma oestradiol between 10 and 18 weeks (Figure 4.7), but plasma progesterone concentration was significantly higher in implanted groups at 10 (before treatment), 12, 14 and 16 weeks ($P < 0.05$, t-test, $n = 24$).

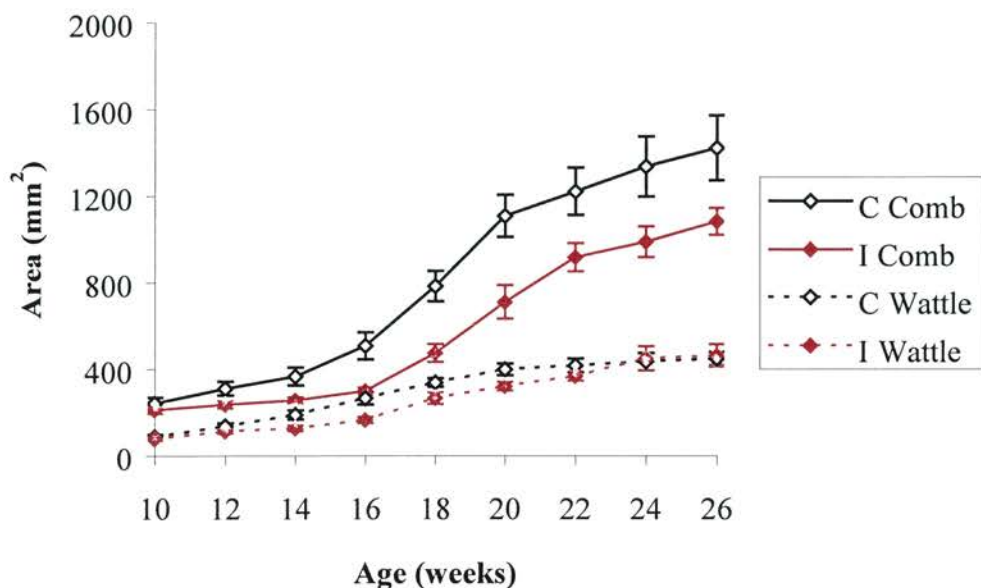


Figure 4.1 Mean comb and wattle growth (\pm SE) between 10 and 26 weeks in 12 groups of pullets treated with oestradiol/progesterone implants (I) or control (C) at 10 weeks of age in Experiment 1a.

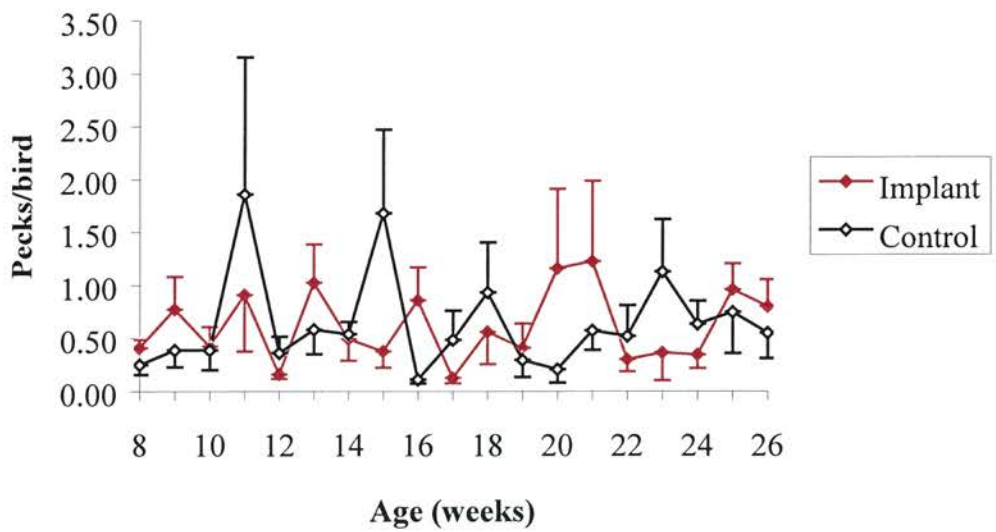


Figure 4.2 Mean numbers of observed gentle pecks given and received per bird (\pm SE) between 8 and 26 weeks of age across groups of implanted or control pullets in Experiment 1a.

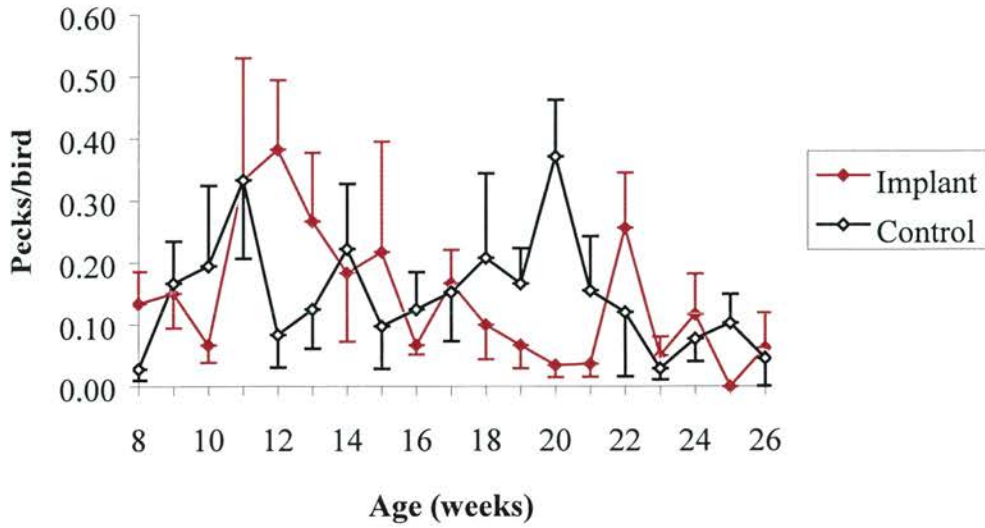


Figure 4.3 Mean numbers of observed vigorous pecks/pulls given and received per bird (\pm SE) between 8 and 26 weeks of age across groups of implanted or control pullets in Experiment 1a.

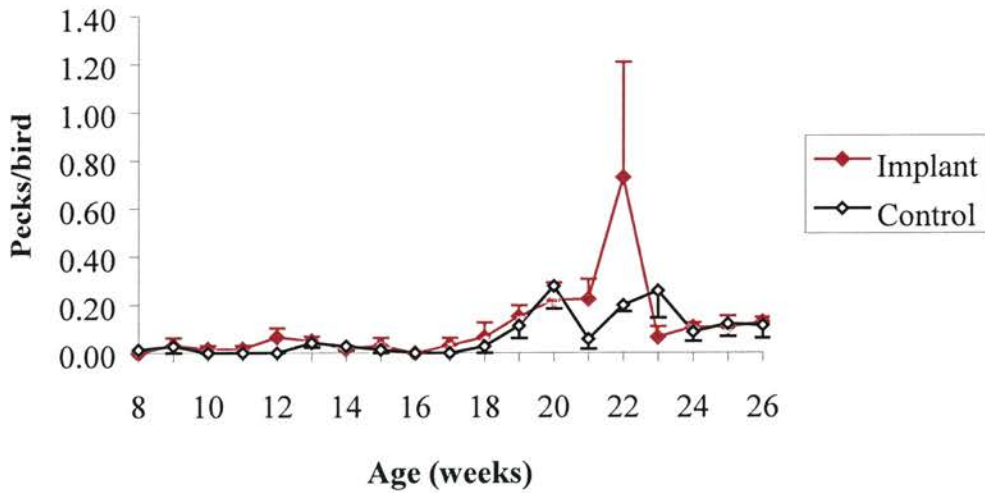


Figure 4.4 Mean numbers of observed aggressive pecks given and received per bird (\pm SE) between 8 and 26 weeks of age across groups of implanted or control pullets Experiment 1a.

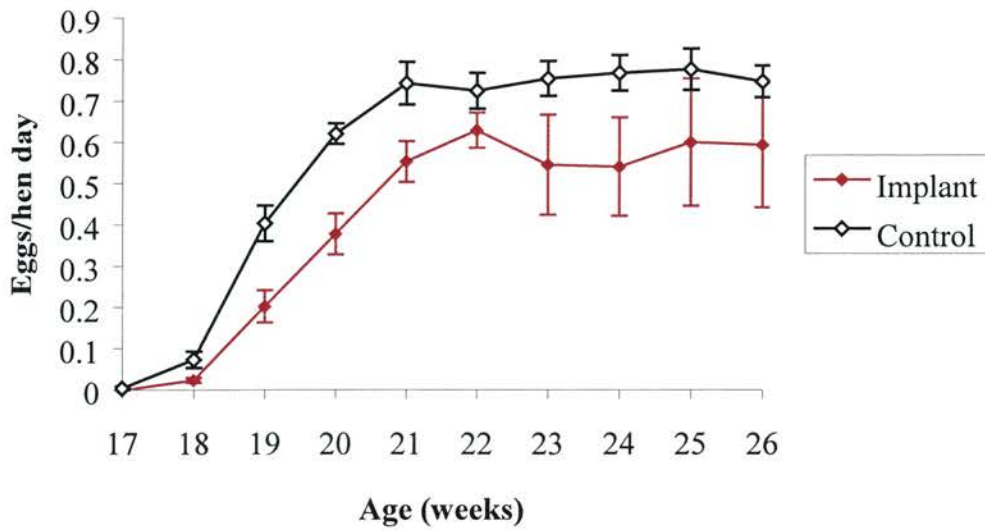


Figure 4.5 Mean total egg production in eggs/hen d (\pm SE) between 17 and 26 weeks of age across groups of implanted or control pullets.

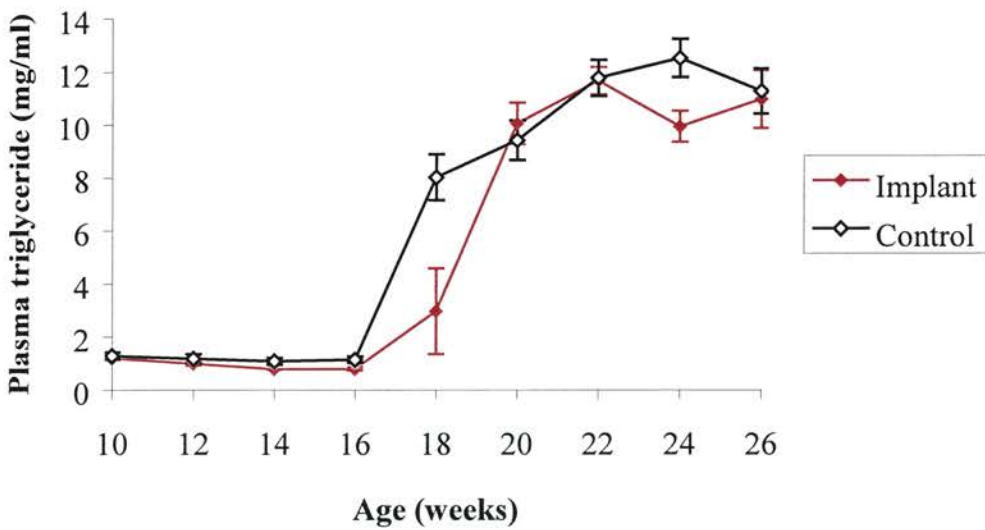


Figure 4.6 Mean (\pm SE) plasma triglyceride (n=12 per treatment at each age) between 10 and 26 weeks of age across groups of implanted or control pullets.

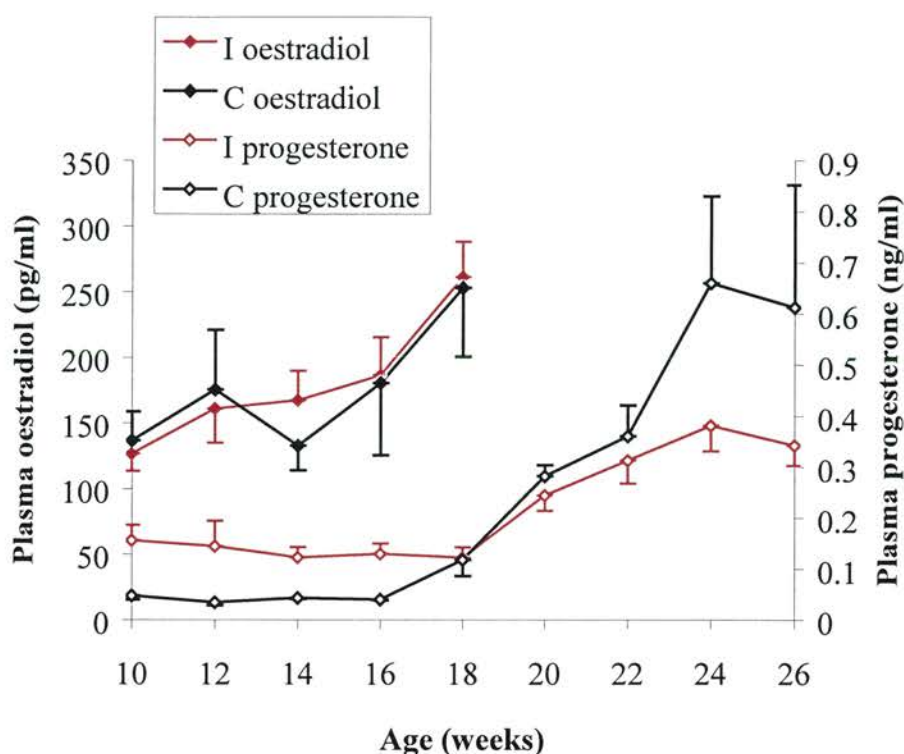


Figure 4.7 Mean (\pm SE) plasma oestradiol and progesterone ($n=12$ per treatment at each age) between 10 and 26 weeks of age across groups of implanted (I) or control (C) pullets in Experiment 1a.

4.2.4 Conclusions

This experiment was unsuccessful because the oestradiol/progesterone implants did not alter hormonal state sufficiently to advance sexual maturation. Implantation of the pellets did not alter plasma oestradiol or triglyceride concentration although plasma progesterone was elevated at 2, 4 and 6 weeks after implantation. There was some evidence that hormone release from the implants did occur in the first two weeks after implantation, as indicated by pecking behaviour and the depression of subsequent egg production, but the sustained release the implants were designed to deliver (60 days) was not apparent.

The failure of the implants to deliver an adequate hormone dose was coupled with a complete absence of damaging feather pecking (although severe aggressive pecking was a problem after the onset of lay in several groups). Hence, few conclusions can be drawn from this study about the effects of implanted gonadal hormones on pecking behaviour.

4.3 Experiment 1b – Pellet investigation

4.3.1 Introduction

Implantation of slow release pellets containing oestradiol and progesterone did not significantly alter reproductive state in Experiment 1a. It is possible that the hormone dose contained in the implants (10mg 17 β -oestradiol and 25mg progesterone) was insufficient, but it was also possible that some aspect of the implantation procedure or the subcutaneous environment was preventing sustained/adequate hormone release. Instructions for use of the pellets stated that “the pellet should not come in contact at the time of implantation with any organic solvent or exogenous fluid”. Therefore, the use of liquid local anaesthetic in Experiment 1a could have altered or impaired implant performance.

To resolve these issues, a small experiment was designed with surplus pellet implants, with the aim of providing more information on hormone release, especially in the first 10 days after implantation. To eliminate possible interference from liquid local anaesthetic, pellets were implanted under general anaesthetic. To determine whether a satisfactory response could be achieved with a higher hormone dose, birds were implanted with 0, 1, 2 or 3 pellets. Rapid encapsulation with connective tissue was also a possible cause of poor release performance, and as such the extent of pellet encapsulation after a 46-day period was examined.

4.3.2 Methods

Twelve 16-week-old Brown leghorn pullets were used in this experiment. These were older than the 10-week-old ISA Brown pullets implanted previously, but were equivalent in terms of bodyweight and stage of maturation. The birds were housed individually in sloping wire floor cages (width 30 cm, height 43-50 cm, depth 46 cm) in the middle row of a three tier battery. The birds were randomly assigned to one of four treatments (0, 1, 2 or 3 pellets), three birds per treatment, and were implanted accordingly with slow release pellet implants under general anaesthetic (see Section 2.10). Blood samples were obtained from all birds on day 0 (before implantation), and days 1, 2, 3, 4, 5, 8, 11, 14, 17, 20, 25, 45 (after implantation). Blood samples were assayed for plasma triglyceride (see Section 2.8). After the

birds were humanely killed at 46 days, the implant sites were examined to determine the degree of pellet encapsulation.

4.3.3 *Results*

The responses of the birds to the implant treatments varied widely. Implantation of one pellet elicited a small increase in plasma triglyceride in the first five days after implantation in two birds, but the third individual showed no response (Figure 4.8b.). Two birds receiving two pellets exhibited large plasma triglyceride responses (up to 25mg/ml) between one and 10 days after implantation, but the third individual did not respond (Figure 4.8c.). Only one of the birds treated with three pellets responded, exhibiting elevated plasma triglyceride between one and 10 days after implantation (Figure 4.8d.). None of the treatments affected plasma triglyceride levels beyond 10 days after implantation. A gradual increase in plasma triglyceride concentration was seen after 20 days in all treatments (including controls) and was related to sexual maturation. Examination of the implant sites after birds were killed at 46 days revealed that the majority of pellets were completely encapsulated in fat and connective tissue.

4.3.4 *Conclusions*

Implantation with one pellet under general anaesthetic did not produce an appreciable change in hormonal state (as in Experiment 1a), eliminating the possibility that use of local anaesthetic interfered with pellet performance in Experiment 1a. Highly variable (and short-lived) responses were observed to double or triple hormone doses; in some birds, treatment with two or three pellets produced plasma triglyceride levels in the range expected for laying hens for up to 10 days, while others did not respond.

The results of this experiment suggest that rapid encapsulation, coupled with a poor and variable rate of release (as opposed to interference from local anaesthetic or insufficient dose) prevent this type of implant being a viable method to reliably deliver steroid hormones in birds.

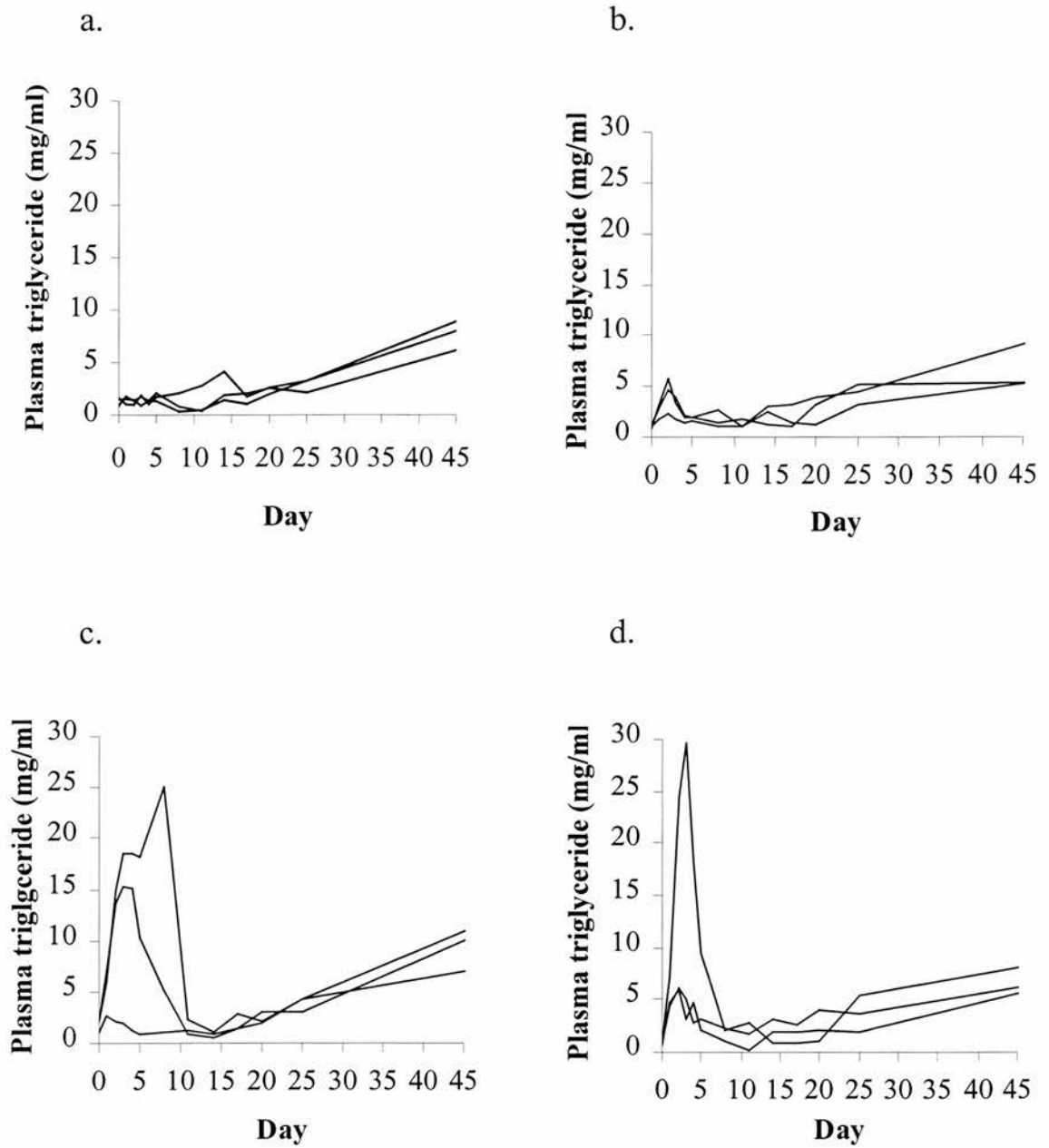


Figure 4.8 Plasma triglyceride profiles (1 to 45 days after implantation) of individual pullets (3 per treatment) treated with different numbers of slow release pellet implants in Experiment 1b; (a.) 0 pellets, (b.) 1 pellet, (c.) 2 pellets (d.) 3 pellets.

4.4 Experiment 2a – First Silastic implant study

4.4.1 Introduction

Silastic implants are a widely used method for chronically administering compounds at physiological concentrations. They consist of lengths of semi-permeable silicon tubing filled with pure crystalline hormone. Body fluids enter the implant and its contents are released at a rate depending on solubility of contents, tubing wall thickness and surface area (Dziuk and Cook, 1966). Silastic implants have been used successfully in several studies to administer gonadal steroids such as oestradiol (Mashaly et al., 1979) progesterone (Johnson, 1983; Saito et al., 1986) and testosterone (Hagen and Dziuk, 1985; Fennell and Scanes, 1992) to birds. This experiment aimed to determine if Silastic implants could be a feasible method of chronic gonadal steroid administration in growing pullets. No published methodology for achieving the required hormone manipulation (advancement of sexual maturity by 4-6 weeks in pullets) is available, so this experiment served as a pilot study to determine the most appropriate implant size and dose to mimic hormonal changes at sexual maturity. The experiment also investigated whether the anti-oestrogen tamoxifen could be reliably administered using Silastic implants, since its use was intended in later studies.

4.4.2 Methods

Fifty ISA Brown pullets were used in this experiment. They were randomly allocated at day old into 10 groups of five birds and were housed and reared in pens to 20 weeks of age as described in Section 2.2. Silastic implants were prepared using 1.5mm lumen diameter Silastic tubing (wall thickness 1mm), and were filled with either a combination of oestradiol and progesterone (pure crystalline hormone in a 50/50 mix, Sigma) or tamoxifen (Sigma) (see Section 2.9). The finished implants were 4 cm long and contained approximately 25mg hormone (Plate 2.1 b).

At 10 weeks of age groups were randomly assigned to one of 5 implant treatments; control (no implants), 1 oestrogen/progesterone combination implant (EP1), 2 oestrogen/progesterone combination implants (EP2), 1 tamoxifen implant (TAM1) or 2 tamoxifen implants (TAM2), 2 groups per treatment. According to treatment, every bird in each group received none, one or two subcutaneous implants

under general anaesthetic (see Section 2.10). Where birds were treated with two implants, one was situated on either side of the neck. Birds and groups were treated in random order. Regular measurements of bodyweight, pecking damage scores (alternate weeks from 8-20 weeks), comb and wattle growth (alternate weeks from 10-20 weeks), and egg production (weekly from 17 to 20 weeks) were carried out as described in Chapter 2. Blood samples were obtained from all birds at 10 weeks (before implantation) and at 11, 12, 14, 15, 16, 17, 18, 19 and 20 weeks. Blood samples were assayed for plasma triglyceride, and a limited number were assayed for oestradiol, progesterone and testosterone (as described in Section 2.8). Plasma testosterone was determined for only one bird in each pen at each age, and the data were pooled from 11-14 and 15-20 weeks for statistical comparison.

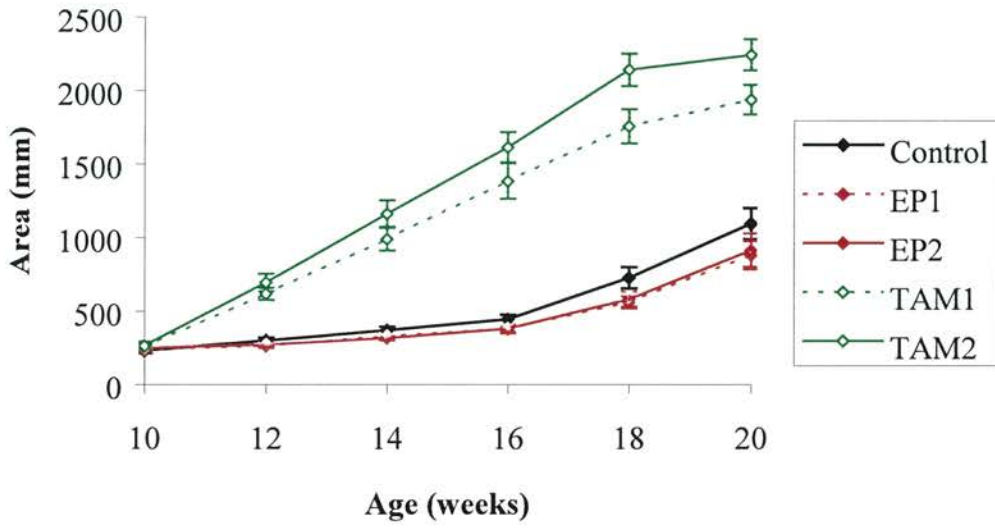
4.4.3 *Results*

Implant treatment had no significant effects on bodyweight, pecking damage scores or egg production at any age (data not shown). Some implant treatments affected comb and wattle growth (Figure 4.9). EP1 treatment had no effect, while EP2 treated birds had significantly smaller wattles than controls at 14, 18 and 20 weeks ($P<0.01$, $P<0.01$ and $P<0.05$, respectively, t-test, $n=20$). The tamoxifen implants had the most pronounced effect on comb and wattle growth, which was significantly increased with both TAM1 and TAM2 treatments at 12, 14, 16, 18 and 20 weeks in comparison to controls (all $P<0.001$, t-test, $n=20$). Birds treated with TAM2 had significantly larger combs at 18 and 20 weeks than those treated with TAM1 ($P<0.05$, t-test, $n=20$).

Between 10 and 16 weeks, plasma triglyceride was not significantly altered by any of the implant treatments (Figure 4.10). At 17 weeks, EP2 treated birds had significantly lower plasma triglyceride than controls ($P<0.05$, t-test, $n=12$). Rapidly increasing plasma triglyceride levels were seen in all treatment groups after 17 weeks, except TAM2 treated birds which had significantly lower plasma triglyceride than controls at 18, 19 and 20 weeks ($P<0.01$, t-test, $n=12$).

Plasma oestradiol, measured between 10 and 14 weeks, was variable between individuals, and although an increase was observed in some EP2 treated birds, there was no significant difference from controls (Figure 4.11).

a.



b.

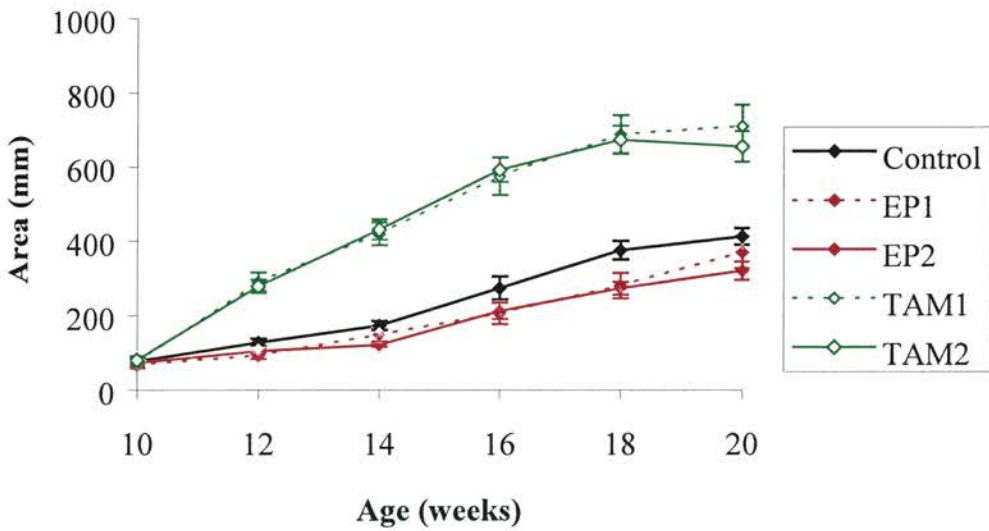


Figure 4.9 Comb (a.) and wattle (b.) growth (\pm SE) between 10 and 20 weeks in pullets treated with one or two Silastic implants containing oestradiol/progesterone (EP1, EP2) or tamoxifen (TAM1, TAM2) at 10 weeks in Experiment 2a.

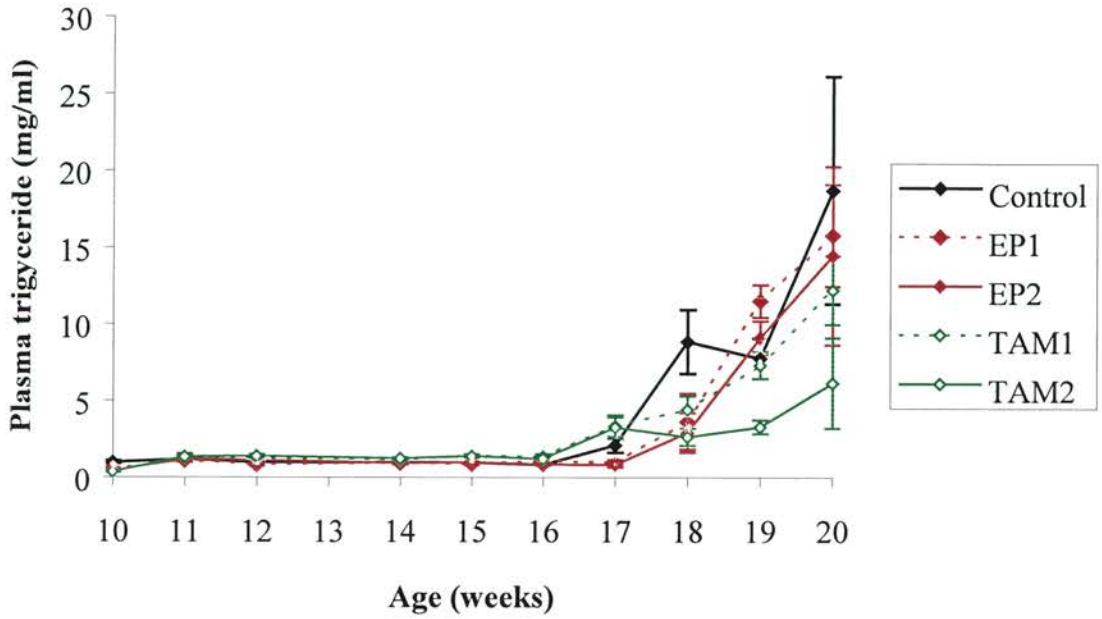


Figure 4.10 Mean plasma triglyceride concentration (\pm SE) ($n=10$ per treatment at each age) between 10 and 14 weeks in pullets treated with one or two Silastic implants containing oestradiol/progesterone (EP1, EP2) or tamoxifen (TAM1, TAM2) at 10 weeks in Experiment 2a.

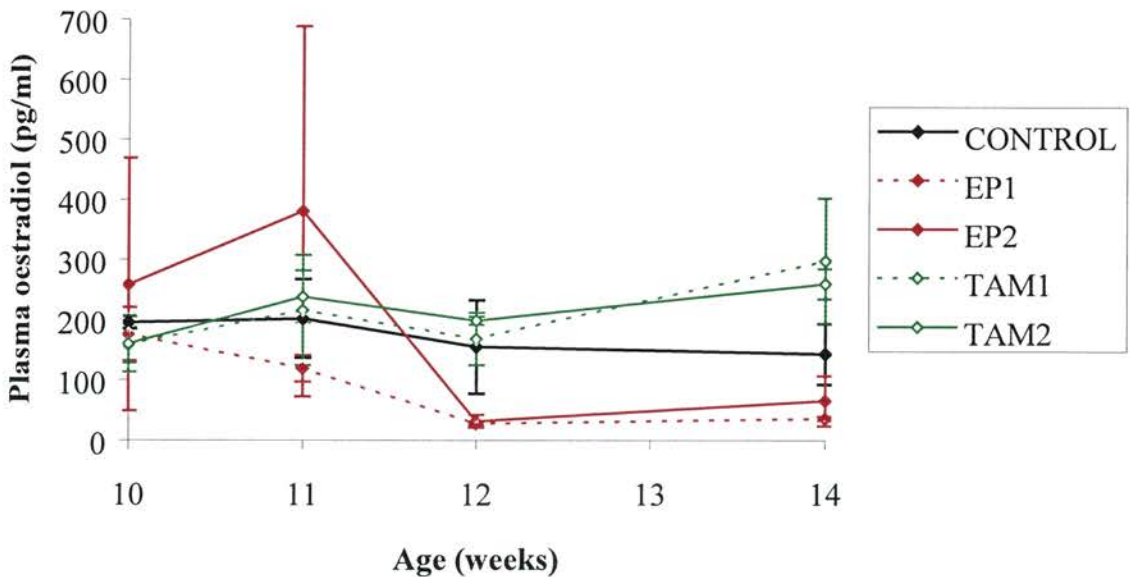


Figure 4.11 Mean plasma oestradiol concentration (\pm SE) ($n=4$ per treatment at each age) between 10 and 14 weeks in pullets treated with one or two Silastic implants containing oestradiol/progesterone (EP1, EP2) or tamoxifen (TAM1, TAM2) at 10 weeks in Experiment 2a.

Plasma progesterone levels were significantly increased with both EP1 and EP2 treatments, with large peaks seen one week after implantation (Figure 4.12). EP1 treatment resulted in significantly elevated plasma progesterone at 11, 12, 16 and 17 weeks compared to controls ($P < 0.001$, $P < 0.01$, $P < 0.05$ and $P < 0.05$ respectively, t-test, $n=8$), while increased plasma progesterone was seen in EP2 treated birds at 11, 12, 14, 15, 16 and 17 weeks ($P < 0.01$, $P < 0.01$, $P < 0.001$, $P < 0.01$, $P < 0.05$ and $P < 0.05$, t-test, $n=8$).

Plasma testosterone was significantly increased by treatment with tamoxifen, with the highest levels seen 5-6 weeks after implantation (Figure 4.13). TAM2 treated birds had higher plasma testosterone compared to controls between 11 and 14 weeks ($P < 0.01$, t-test, $n=12$), and both TAM1 and TAM2 treatments caused elevated plasma testosterone between 15 and 20 weeks ($P < 0.05$, $P < 0.001$ respectively, $n=16$).

4.4.4 *Conclusions*

One week after implantation, plasma triglyceride was not elevated in birds receiving oestradiol/progesterone combination implants, suggesting that the implants were not delivering an appreciable amount of oestradiol. The implants did deliver progesterone, plasma levels of which increased in a dose dependant manner in some birds, although responses varied. Release of tamoxifen from the implants also appeared to be successful, and tamoxifen treated birds at both doses were characterised by increased rates of comb and wattle growth. These effects were probably due to increased endogenous testosterone production, which responded to tamoxifen in a dose dependant way. The hormonal manipulations achieved did not affect pecking behaviour (as reflected by pecking damage scores) or egg production. Despite the failure of the implants to deliver oestradiol as effectively as the other compounds, these results confirm that Silastic implants have potential as a tool to chronically manipulate hormonal state in pullets.

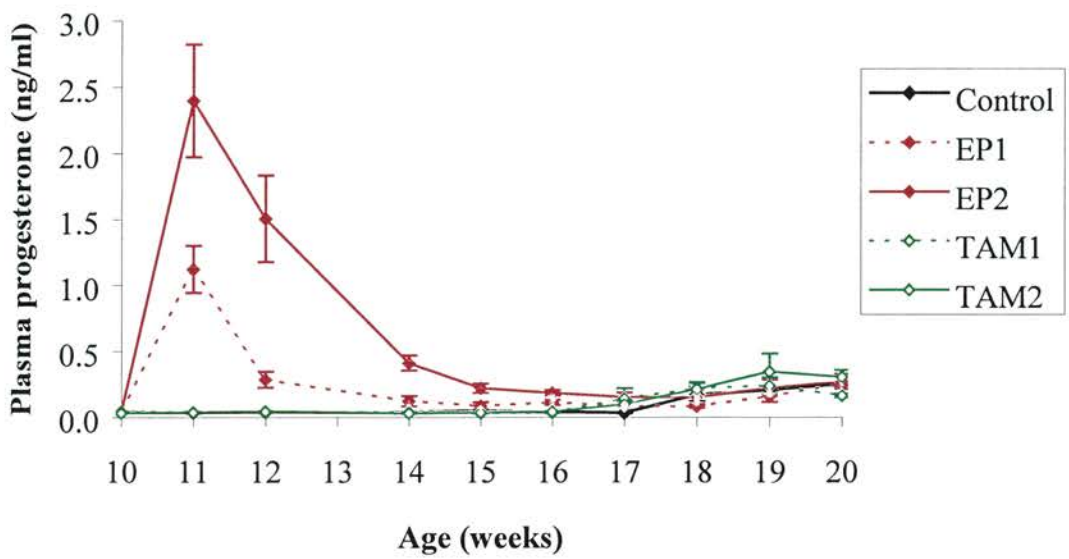


Figure 4.12 Mean plasma progesterone concentration (\pm SE) (n=4 per treatment at each age) between 10 and 20 weeks in pullets treated with one or two Silastic implants containing oestradiol/progesterone (EP1, EP2) or tamoxifen (TAM1, TAM2) at 10 weeks in Experiment 2a.

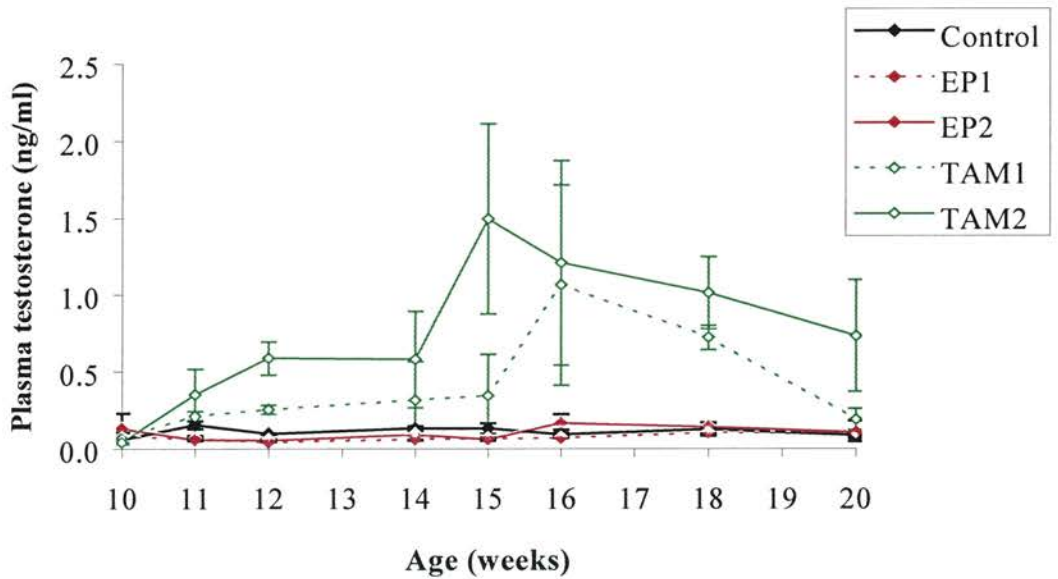


Figure 4.13 Mean plasma testosterone concentration (\pm SE) (n=2 per treatment) between 10 and 20 weeks in pullets treated with one or two Silastic implants containing oestradiol/progesterone (EP1, EP2) or tamoxifen (TAM1, TAM2) at 10 weeks in Experiment 2a.

4.5 Experiment 2b – Second Silastic implant study

4.5.1 Introduction

Silastic implants containing an oestradiol/progesterone mixture did not successfully deliver oestradiol in Experiment 2a. However, a definite release of progesterone was detected, raising the question of whether the two substances were affecting each other's release when placed in the same implant. To investigate this possibility, an experiment was designed to administer oestradiol and progesterone in separate implants. Since there was also a possibility that some physical aspect of the implants used in Experiment 2a had impaired the release of oestradiol, the Silastic implants constructed for the second experiment were thinner walled than before and were longer to provide a greater surface area.

4.5.2 Methods

Twenty-four ISA Brown pullets were used in this experiment, housed individually in sloping wire floor cages (width 30 cm, height 43-50 cm, depth 46 cm) in the middle row of a three tier battery. Separate oestradiol and progesterone Silastic implants were constructed from thin walled Silastic tubing (0.23 mm wall thickness, 1.5mm lumen diameter)(see Section 2.9), and were 9cm long (Plate 2.1 a). The increased length of these implants made them potentially difficult to implant, so they were folded in half and tied with suture (Braided sterile suture, Ethicon) before implantation.

Birds were randomly assigned to one of six treatments: control (no implants (C)), one oestrogen implant (E1), two oestrogen implants (E2), one progesterone implant (P1), two progesterone implants (P2) or one oestrogen implant + one progesterone implant (EP), four birds per treatment. According to treatment, birds received none, one or two subcutaneous implants under general anaesthetic (see Section 2.10) at 10 weeks of age. Where birds were treated with two implants, one was situated on either side of the neck. Birds were treated in random order.

Blood samples were obtained from all birds at day 0 (before implantation) and at 6, 12, 24, 36, 48 days after implantation. Blood samples were assayed for

plasma triglyceride, and a limited number were assayed for progesterone (birds receiving C, P1, P2 and EP treatments).

4.5.3 *Results*

Plasma triglyceride levels were significantly increased by E2 treatment at 6, 12 and 24 days after implantation (all $P < 0.05$, t-test, $n=8$), indicating that release of oestradiol from the implants had taken place (Figure 4.14). Levels of plasma triglyceride in E1, P1, P2 and EP treated birds did not significantly differ from controls. Plasma progesterone levels were greatly elevated in P2 treated birds (up to 5ng/ml), and slight increases were seen in P1 and EP treated birds compared to controls (Figure 4.15). Treatment effects on plasma progesterone could not be confirmed statistically because of the small number of observations ($n=2$ at each age for each treatment).

4.5.4 *Conclusions*

Changes in plasma triglyceride indicated that release of oestradiol from the implants had taken place. However, treatment with one oestradiol implant had no significant effects and treatment with two implants produced only a small increase in plasma triglyceride in the first 12 days after implantation, with responses varying widely between individuals. The implants delivered progesterone successfully, with plasma progesterone levels typical of onset of lay (c.1ng/ml) being observed when birds were treated with one implant, and higher plasma levels when birds were treated with two implants.

While Silastic implants were effective in administering tamoxifen and progesterone, they proved unreliable for delivering oestradiol. Adequate, reliable release of oestradiol is vital to mimic the hormonal state of sexually maturing pullets, and minimal variation between individuals is important because of possible effects of individual variation in plasma hormone levels on behaviour. In this experimental context, these requirements were not met by Silastic implants for the administration of oestradiol.

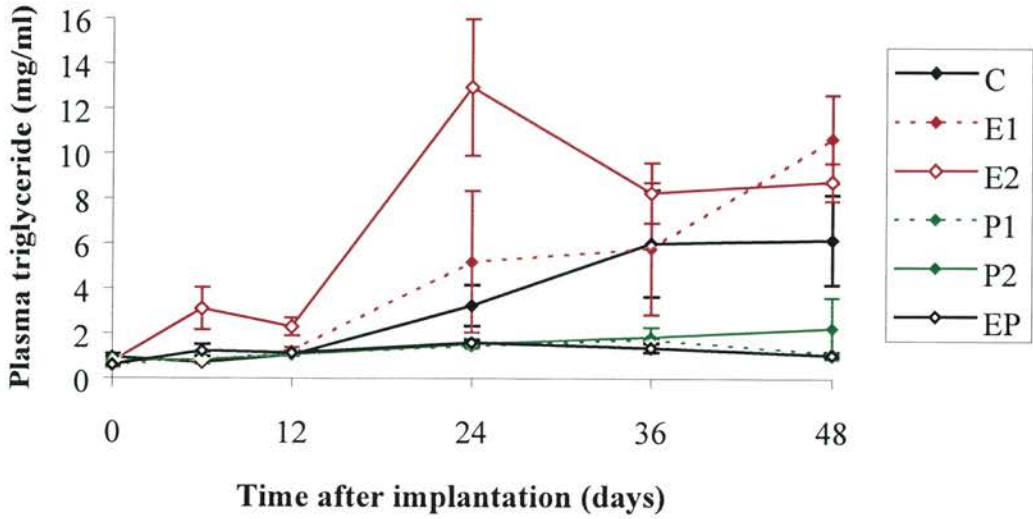


Figure 4.14 Mean plasma triglyceride concentration (\pm SE) ($n=4$ per treatment at each age) in pullets between 0 and 48 days after implantation with one or two Silastic implants containing oestradiol (E1, E2), progesterone (P1, P2) or both (EP) at 10 weeks of age in Experiment 2b.

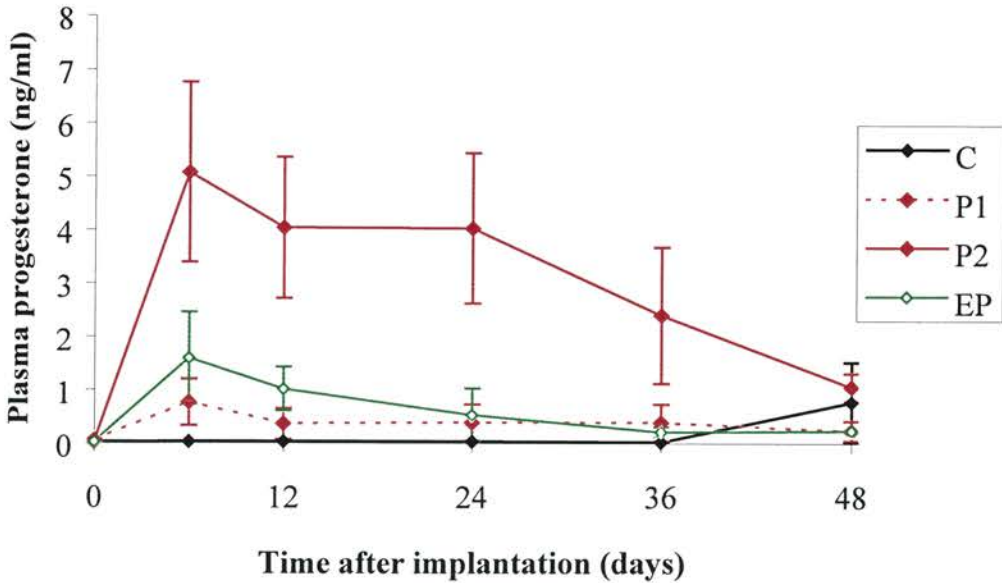


Figure 4.15 Mean (\pm SE) plasma progesterone ($n=2$ per treatment at each age) in pullets between 0 and 48 days after implantation with one or two Silastic implants containing progesterone (P1, P2) or both oestradiol and progesterone (EP) at 10 weeks of age in Experiment 2b.

4.6 Experiment 3 – Oral administration

4.6.1 Introduction

Administering compounds orally via food or drinking water is advantageous in that it is non-invasive, reliable and is easily applied to large numbers of animals. For these reasons, oral administration of agents such as antibiotic growth promoters and coccidiostats to growing birds is used routinely in the poultry industry. Experimentally, various compounds have been administered to poultry via food or drinking water, such as mineral salts (Khalafalla et al., 1998), vitamin C (Pardue et al., 1993), melatonin (Noddegaard and Kennaway, 1999) the anti-oestrogen tamoxifen (Hiura, 1990), the anti-progesterone RU-38486 (Bar et al., 1996), non-steroidal oestrogens and testosterone (Malik and Aggarwal, 1970).

The oral bioavailability of gonadal steroids in humans and laboratory animals has been studied extensively in relation to the development of oral contraceptives (Lobo and Cassidenti, 1992; Nahoul et al., 1993; Jarvinen et al., 1999). However, no such information is available for oral administration of steroid hormones to birds. This experiment investigated the viability of a novel method of oral administration of oestradiol and progesterone to pullets. Any strategy for oral administration of gonadal steroids must be via food, because low solubility precludes inclusion of steroids in drinking water. Unfortunately, however, the addition of steroid hormones directly to food also poses problems such as cross contamination between treatments and serious human safety risks. Gao and Short (1994a, 1994b) offered mice paraffin wax blocks containing cereal grains and the antiprogestosterone RU486 or methyl testosterone. In free feeding trials, the mice ingested the wax and absorbed its contents, which successfully controlled fertility. In the current study, this approach was adapted for use with pelleted poultry diet. This involved coating food pellets in paraffin wax dosed with hormone, the wax serving to seal the steroid in the food and reduce contamination. The required hormone dose was achieved by measuring daily food intake and preparing food dosed accordingly. The experiment was a pilot study, with the aim of determining whether pullets would eat wax-coated food, and if so, to what extent the gonadal hormones oestradiol and progesterone are absorbed.

4.6.2 *Methods*

Food intake was measured in 10-week-old ISA Brown pullets and was found to be approximately 100g/d. The intended dose of oestradiol and progesterone was 5mg/d, so experimental diets were prepared containing 5mg hormone per 100g food. Treatment diets were prepared using normal layer grower pellets (3mm diameter, 164 g/kg CP, 11.8 MJ/kg ME) in 500g batches as follows. Paraffin wax (50ml) was melted in a glass beaker on a hot plate, 25mg of the appropriate crystalline steroid (17 β -oestradiol or progesterone, Sigma) was added, and the mixture was stirred for five minutes with a magnetic stirrer. Layer grower pellets (500g) were placed in a large plastic beaker and warmed in a microwave on 'high' for one minute (stirring once after 30 seconds). The molten wax was added to the warmed food and stirred vigorously until all the pellets were coated. The wax-treated food was placed in plastic bags to cool, and stored until required. Three different diets were prepared in this way; control (wax coated, no hormone added), oestradiol (5mg/100g) and progesterone (5mg/100g).

Nine, 10-week-old ISA Brown pullets were randomly assigned to control, oestradiol or progesterone diet treatments (three birds per treatment) for 15 days. The birds were housed in individual, alternate sloping wire floor cages (width 30 cm, height 43-50 cm, depth 46 cm) in the middle row of a three tier battery. The diets were presented in high-sided food dishes to ensure birds could not reach the food of their neighbour. Blood samples were obtained from every bird after 2, 4, 6, 9, 12, and 15 days of dietary treatment. The blood samples were assayed for plasma triglyceride, oestradiol and progesterone.

4.6.3 *Results*

Food intake was significantly reduced in the first three days of dietary treatment from 92.0 ± 12.7 g to 70.3 ± 5.8 g ($P < 0.01$, t-test, $n = 18$), but returned to pre-treatment levels within four days. All birds gained weight during the 15 day experiment (mean weight gain 293 ± 35.3 g). Plasma triglyceride concentration became elevated after four days of dietary treatment (increasing from 2-3 mg/ml to c.12mg/ml) in two out of three birds receiving the oestradiol diet (Figure 4.16). However, there was no evidence of elevated plasma oestradiol in birds receiving the

oestradiol treated diet (Figure 4.17). Plasma progesterone concentration increased temporarily in only one bird fed the progesterone treated diet, accounting for the peak seen in the mean response at 9 days (Figure 4.17).

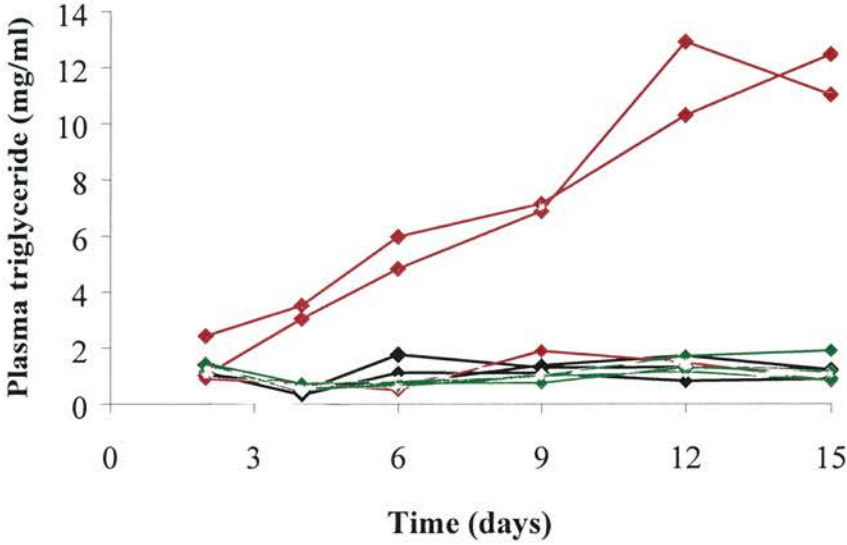


Figure 4.16 Plasma triglyceride concentration in nine individual 10-week-old pullets fed control (black), oestradiol (red) or progesterone (green) wax-coated diets for 15 days in Experiment 3.

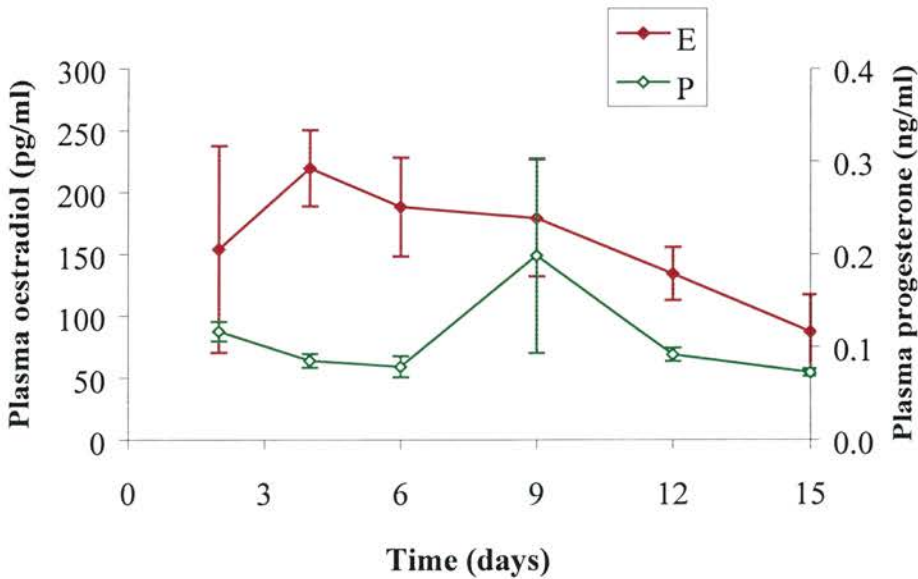


Figure 4.17 Mean (\pm SE) plasma oestradiol and progesterone ($n=3$ each time point) in pullets fed oestradiol (E) or progesterone (P) wax-coated diets for 15 days in Experiment 3.

4.6.4 *Conclusions*

After initial aversion, the pullets ate the wax-coated food and normal weight gains in all individuals demonstrated that the wax treatment had no anti-nutritive properties. Increased plasma triglyceride concentrations were observed in two out of three birds in the oestradiol treatment group, suggesting that the hormone was being absorbed. However, there was no evidence of systemic increases in either oestradiol or progesterone concentrations when they were measured in plasma. The most likely explanation for the increased plasma triglyceride levels observed is that oestradiol was being absorbed and broken down in the liver (with concomitant production of triglyceride). It would seem that even at extremely high doses, steroids administered orally by this method are not well absorbed and are metabolised quickly by the liver, preventing circulating levels from being significantly altered.

4.7 Experiment 4 – Effects of acute tamoxifen administration on novel object pecking

4.7.1 *Introduction*

An alternative approach to the manipulation of hormonal state is the application of antagonists, which reverse or block hormone effects. Tamoxifen, a triphenylethylene anti-oestrogen, is a very potent antagonist of oestrogen in the chicken, and exhibits very little agonist activity compared to its effects in other species (Lazier, 1987). If an oestradiol receptor mediated relationship exists between pecking behaviour and oestradiol, we would expect pecking behaviour to be altered by tamoxifen.

In this experiment, the effects on pecking behaviour of acute administration of tamoxifen in individually housed hens previously identified as ‘peckers’ or ‘non-peckers’ was determined. It has been suggested that feather pecking in hens is associated with pecking at inanimate objects (Channing et al., 1998; Blokhuis and Beuving, 1993), and in this study pecks directed at a novel pecking device were used as an estimate of propensity to peck. The pecking device presented was a bunch of white string, a stimulus which can be pecked or pulled and has been shown to elicit pecking in hens previously (Jones and Carmichael, 1998).

4.7.2 *Methods*

Sixteen 42-week-old ISA Brown hens were used in this study, which had previously been used in the dietary protein source experiment described in Chapter 5, and were also subjected to behavioural tests examining feather eating behaviour (see Chapter 6, Experiments 4a and 4b) before the current study. The birds were defined as ‘peckers’ (birds from pens where pecking damage had taken place and which were known from direct observation to engage in damaging bird to bird pecking) or ‘non-peckers’ (individuals selected at random from groups where no pecking damage had taken place). Of the eight non-peckers, four had previously been on the plant protein treatment and four had been on the animal protein treatment. Of the eight peckers, five had previously been on the plant protein treatment and three had been on the animal protein treatment. The birds were housed individually in sloping wire floor cages (width 30 cm, height 43-50 cm, depth 46 cm) in the middle row of a three tier battery. Birds were housed in alternate cages, so they had visual, but not physical access to their neighbours. *Ad libitum* access to food (standard layer pellets) and water was provided via a food trough at the front of the cage and nipple drinkers to the rear of the cage.

Birds were randomly allocated to tamoxifen or control treatment in a four by four design, with four peckers and four non-peckers receiving each treatment. Tamoxifen treatment birds received three 1ml intramuscular injections (in the thigh) of 2mg/kg body weight tamoxifen in olive oil on three consecutive days (days 0, 1 and 2). Control birds received identical injections of vehicle alone. Blood samples were obtained from every bird in four time periods; 0 (pre-treatment), 1 (immediately after treatment, day 3), 2 (1 week later, day 10) and 3 (another week later, day 17). The blood samples were assayed for triglyceride, oestradiol and testosterone (as described in Section 2.8). Egg production was monitored daily from the first day of treatment injections.

Propensity to peck a novel pecking device was determined on four occasions, in time periods 0, 1, 2 and 3. The pecking device consisted of a bunch of eight equal length (10cm) strands of white string (woven polypropylene twine), held together at one end with 1cm wide white tape. The taped end was attached to a 12cm length of thick, clear fishing line, which in turn was tied to a small (3 x 4 cm) bulldog clip, so

that the total length of the device was 25 cm. When testing, the device was clipped to the floor of the top tier cage above the test hen, so that it hung freely in front of and at the mid-point of the test hen's cage. Each hen was presented with the pecking device for 10 min on three consecutive days, during which time numbers of gentle pecks, gentle pulls, vigorous pecks and vigorous pulls at the device were recorded by an observer sitting adjacent to and 1m to the left of the test cage. Hens were tested in random order, and all tests took place in the afternoon to minimise any effect of oviposition. Counts of gentle pecks, gentle pulls, vigorous pecks and vigorous pulls were summed across the three tests carried out in each time period for each bird. Pre-treatment testing was the first occasion the birds had encountered the pecking device, and the device was removed between tests.

Total counts of gentle pecks, gentle pulls, vigorous pecks and vigorous pulls for each time period were transformed (natural log (count +1)), and split plot ANOVAs were carried out to determine effects of time (time period), treatment (tamoxifen/control), pecking status (pecker/non-pecker) and their interaction. Because of significant pre-treatment differences, the analysis was repeated including only time periods 1, 2 and 3 to examine post-treatment effects. Effects of treatment on egg production and plasma parameters in each time period were determined by t-test.

4.7.3 *Results*

Egg production was significantly reduced by tamoxifen treatment ($P < 0.01$, t-test, $n=16$) with cessation of egg laying for up to 12 days observed in six out of eight tamoxifen treated birds (Figure 4.18).

Tamoxifen treatment resulted in significant reduction in plasma triglyceride concentration in time periods 1 and 2 (immediately after treatment and 10 days after treatment began, $P < 0.001$ and $P < 0.05$ respectively, t-test, $n=16$), but returned to normal by time period 3 (Figure 4.19). There were no differences in plasma triglyceride between peckers and non-peckers within treatments.

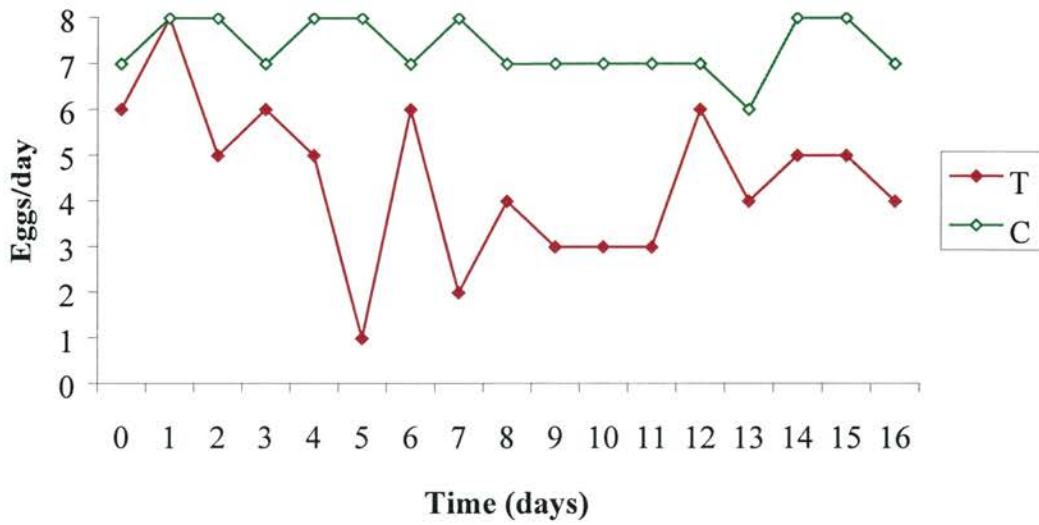


Figure 4.18 Daily egg production in groups of 8 hens during (days 0-2) and after (days 4-17) treatment with tamoxifen (T) or control (C) in Experiment 4.

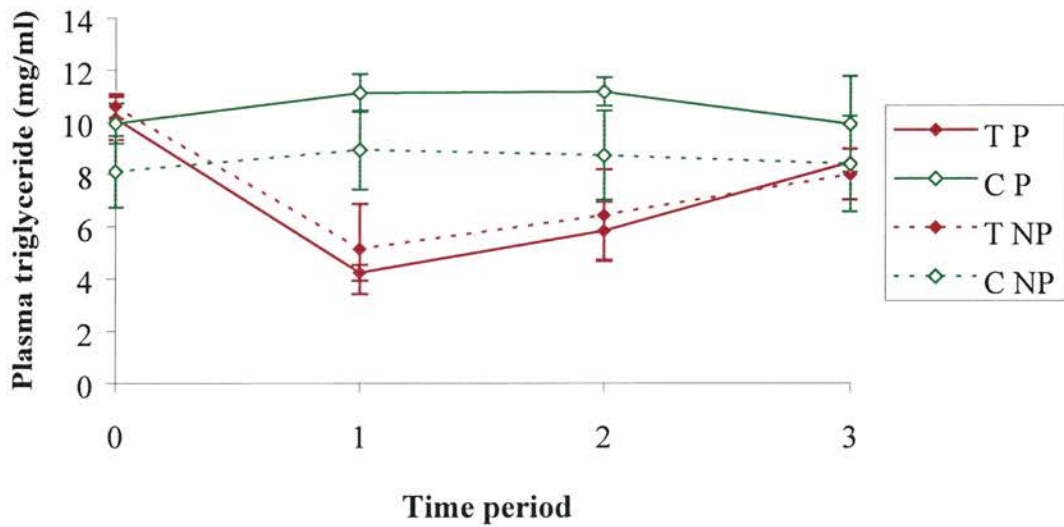


Figure 4.19 Mean plasma triglyceride concentration (\pm SE, $n=4$) across four time periods in peckers (P) and non-peckers (NP) treated with tamoxifen (T) or control (C) in Experiment 4.

Treatment with tamoxifen significantly increased plasma oestradiol in time periods 1 and 2 (both $P < 0.01$, t-test, $n=8$, Figure 4.20). Peckers and non-peckers responded similarly, and there were no significant within-treatment differences. Plasma testosterone also increased in tamoxifen treated birds in time periods 1 and 2 ($P < 0.01$, t-test, $n=8$, Figure 4.21). Within the tamoxifen treatment, peckers had significantly higher plasma testosterone than non-peckers ($P < 0.05$, t-test, $n=8$) in time period 3.

Both peckers and non-peckers readily pecked and pulled the pecking device, with gentle pulls seen most often, followed by vigorous pecks and pulls, then gentle pecks. There were significant time, treatment and pecking status effects on counts of pecks and pulls at the pecking device. Significant pre-treatment differences were observed between peckers and non-peckers, with non-peckers gently pecking the string bunch more than peckers ($P < 0.05$). Peckers allocated at random to the control or tamoxifen treatments also differed in their propensities to vigorously peck and pull the pecking device in the pre-treatment period, with control peckers vigorously pecking and pulling less than tamoxifen peckers ($P=0.053$).

During the post-treatment periods only, there were significant effects of time, but not treatment or pecking status, on numbers of gentle pecks and pulls (both $P < 0.001$). Gentle pecks tended to decrease with time (Figure 4.22), while gentle pulls, which were seen more often, remained reasonably constant (Figure 4.23). There were significant time-treatment interactions in numbers of vigorous pecks (Figure 4.24) and pulls (Figure 4.25) (both $P < 0.05$). Tamoxifen treated birds, regardless of pecking status, exhibited a reduction in vigorous pecking and pulling in time period 1 followed by an increase in time period 2, when numbers of both vigorous pecks and pulls were significantly higher in tamoxifen treated birds than controls. There was no such difference in time period 3.

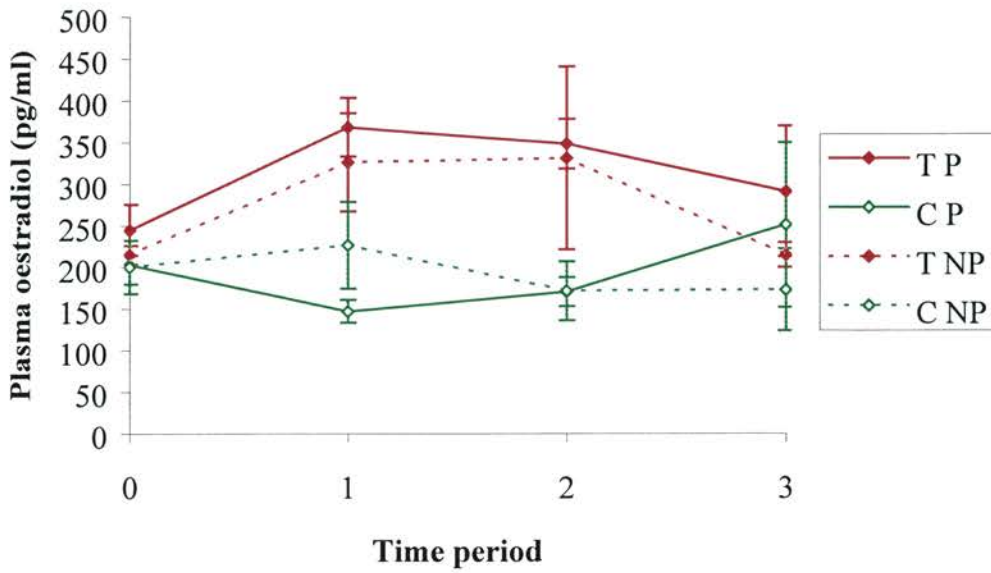


Figure 4.20 Mean plasma oestradiol concentration (\pm SE, $n=4$) across four time periods in peckers (P) and non-peckers (NP) treated with tamoxifen (T) or control (C) in Experiment 4.

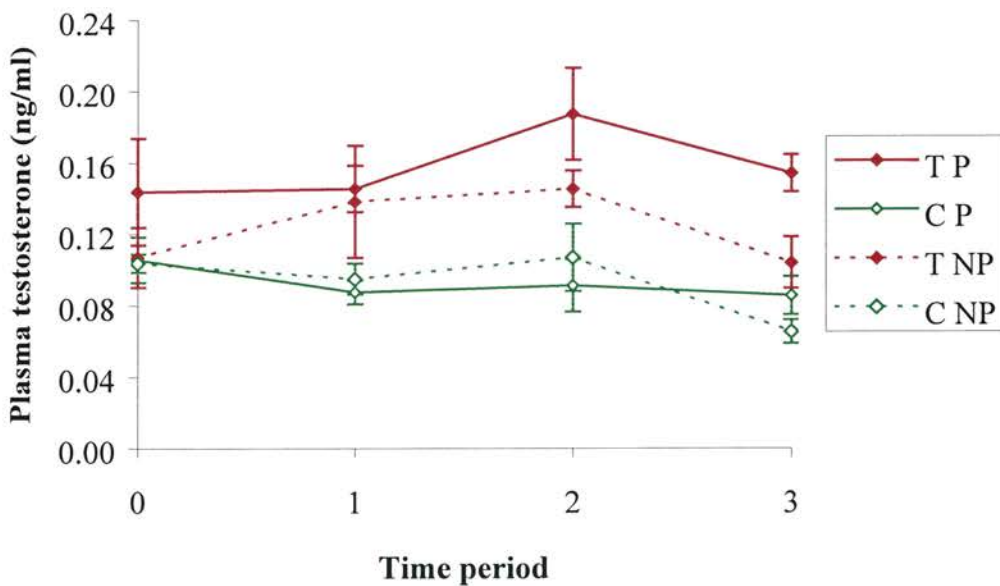


Figure 4.21 Mean plasma testosterone concentration (\pm SE, $n=4$) across four time periods in peckers (P) and non-peckers (NP) treated with tamoxifen (T) or control (C) in Experiment 4.

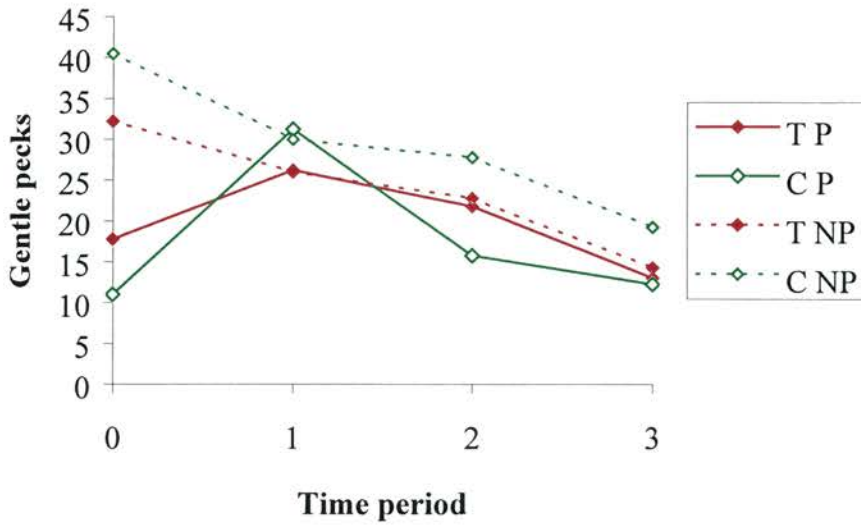


Figure 4.22 Mean numbers of gentle pecks at a bunch of string in four time periods (days 0 (pre-treatment), 3, 10 and 17) in peckers (P) and non-peckers (NP) treated with tamoxifen (T) or control (C) in Experiment 4.

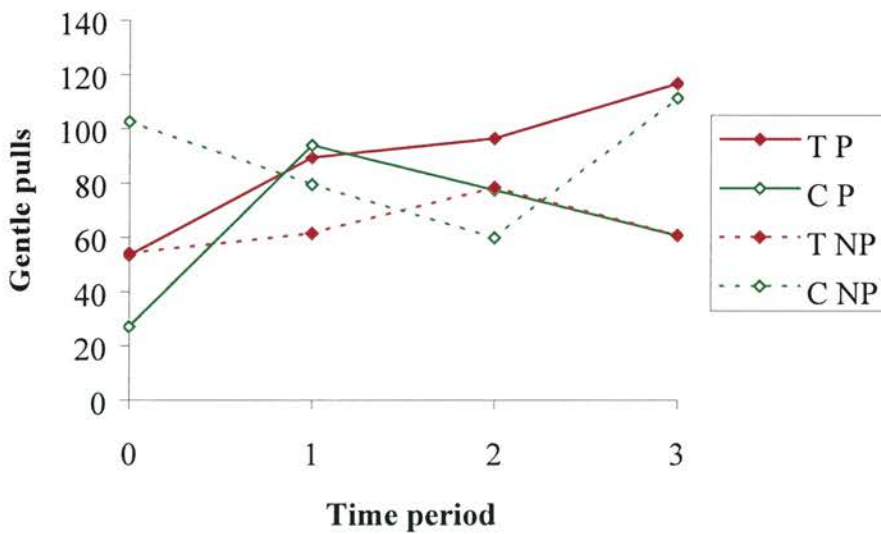


Figure 4.23 Mean numbers of gentle pulls at a bunch of string in four time periods (days 0 (pre-treatment), 3, 10 and 17) in peckers (P) and non-peckers (NP) treated with tamoxifen (T) or control (C) in Experiment 4.

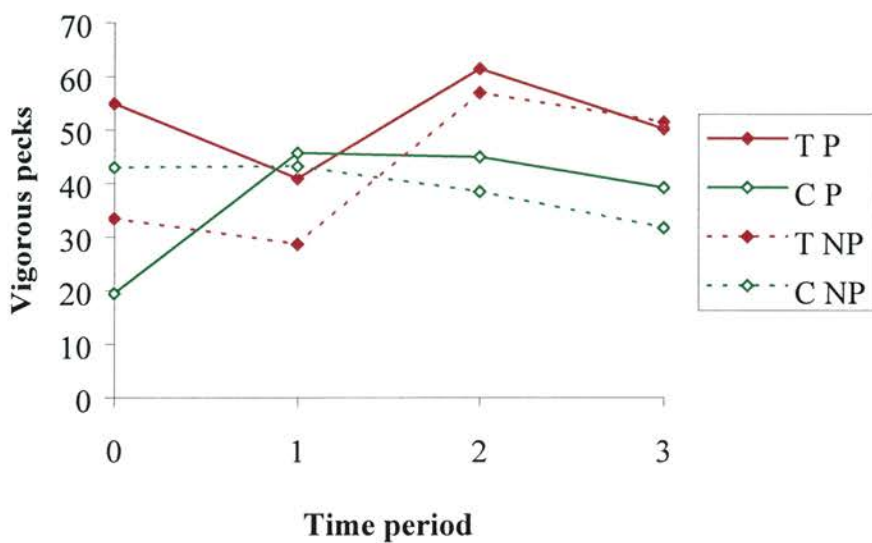


Figure 4.24 Mean numbers of vigorous pecks at a bunch of string in four time periods (days 0 (pre-treatment), 3, 10 and 17) in peckers (P) and non-peckers (NP) treated with tamoxifen (T) or control (C) in Experiment 4.

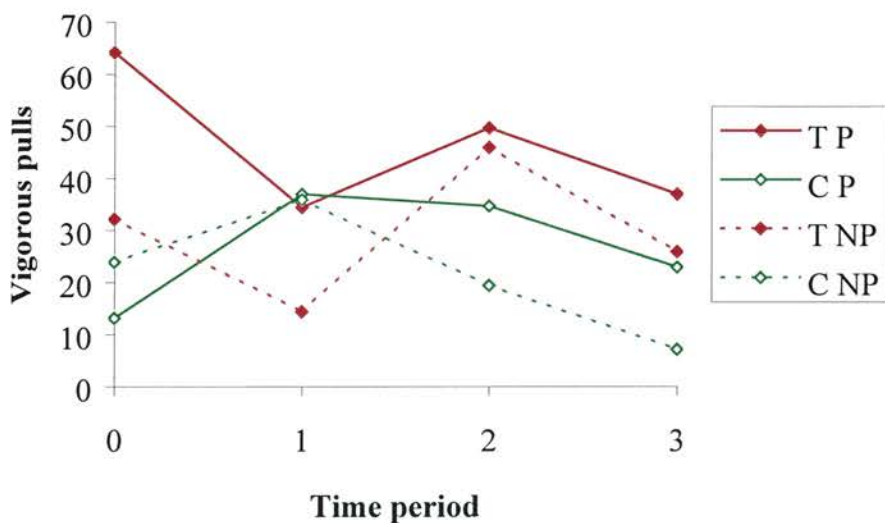


Figure 4.25 Mean numbers of vigorous pulls at a bunch of string in four time periods (days 0 (pre-treatment), 3, 10 and 17) in peckers (P) and non-peckers (NP) treated with tamoxifen (T) or control (C) in Experiment 4.

4.7.4 *Conclusions*

There was no evidence of any difference in basal plasma concentrations of oestradiol or testosterone between peckers and non-peckers. Before treatment with tamoxifen or control, peckers gently pecked the pecking device less than non-peckers, but peckers and non-peckers did not differ in their propensity to peck or vigorously pull the device. Administration of tamoxifen resulted in reduced plasma triglyceride and elevated plasma oestradiol and testosterone in peckers and non-peckers. Regardless of pecking status, treatment with tamoxifen significantly reduced numbers of vigorous pecks and pulls at the pecking device immediately after treatment. Ten days after treatment, numbers of vigorous pecks and pulls were higher in tamoxifen treated birds than controls. These results support the notion of a causal link between potentially damaging pecks and pulls (at a pecking device in this experiment) and oestradiol.

4.8 **Discussion**

The original aim, to repeat and extend the work carried out by Hughes (1973), and examine in detail the effects of gonadal hormones on bird to bird pecking, was not achieved, because reliable, chronic hormone administration proved difficult. Investigation of a number of hormone administration techniques, including two types of implant and oral administration, failed to eliminate problems such as inadequate elevation of circulating hormone levels, and a high degree of variation in response between treated individuals. The reasons for the variability observed between individuals in implant performance are not known. Possibilities include variation in initial positioning of the implant (and therefore proximity to local circulation) or subsequent movement of the implant in the subcutaneous environment. The reaction of individual birds to the introduction of a foreign body could also have resulted in differing rates of encapsulation.

The slow release pellet implants used in Experiments 1a and 1b did not perform according to the manufacturer's specifications. The reasons for the poor performance of the implants are not fully understood, but would appear to be a combination of poor release and rapid encapsulation. It is possible that some aspect of the avian subcutaneous environment impaired pellet performance, illustrating that

caution should be exercised when transferring techniques designed for use in mammals to birds. Only one previous study (Gordon et al., 1988) has reported using these slow release pellets in birds, in which implants containing 100mg oestradiol were administered to cockerels, to provide release over 14 days. In that study, the implants produced responses (mRNA destabilisation) indicative of oestradiol release, although the investigators did not mention the site of implantation and made no attempt to ascertain rate of release. This suggests that pellet implants can be effective in some situations, particularly in studies involving larger doses and shorter time scales.

In the current studies, there was some evidence of hormone release by the pellets. In Experiment 1a, plasma progesterone was elevated in implanted birds for up to six weeks after implantation (although it should be noted that plasma progesterone was significantly higher in the randomly allocated implant groups *before* implantation). Plasma triglyceride and oestradiol were unaffected by implant treatment, at least by the time the first post-treatment blood sample was collected, two weeks after implantation. The results of Experiment 1b (where a triglyceride response to pellet implantation was seen in some individuals up to 10 days after implantation) suggest that any oestradiol/triglyceride response was probably 'missed' by the sampling regime employed in Experiment 1a. The metabolic clearance rates of oestradiol and progesterone are similar (73.7 and 73.8ml plasma/min/kg respectively, Johnson and van Tienhoven, 1981; Tsang and Grunder, 1984), so it would appear that increased release of progesterone accounted for the higher plasma levels observed, as opposed to a more rapid clearance of oestradiol.

Although the results of Experiment 1b suggested that any oestradiol release from the pellets was short-lived, prolonged effects of implant treatment were seen in Experiment 1a, namely delayed comb and wattle growth, depressed egg production and lower plasma triglyceride up to 16 weeks after implantation. The effects on comb and wattle growth and egg production observed were likely to be due to the effects of the negative feedback mechanism between steroids and gonadotrophins, in which inhibition of lutenizing hormone (LH) secretion by oestradiol and progesterone reduces further secretion of steroids (Etches, 1996). Although plasma testosterone was not measured, it is likely that suppression of testosterone production

by LH resulted in the delayed comb and wattle growth seen in implanted birds. Negative effects on egg production of steroid administration have been demonstrated in several studies, particularly with progesterone treatment (Cook and Warnik, 1962; Hagan et al., 1984; Saito et al., 1986) but also with oestradiol (Allee and Collias, 1940). It would appear that the doses of oestradiol and progesterone administered by the pellets were not high enough to act directly on target tissues to advance maturation, but were sufficient to disrupt the gonadotrophin feedback mechanism and reduce subsequent secretion of gonadal steroids, thereby delaying sexual maturity.

A complete lack of damaging pecking in Experiment 1a makes it very difficult to draw any conclusions about the effects on pecking behaviour of the hormonal manipulations achieved. Numbers of vigorous pecks were higher in implanted groups at 12 weeks of age (two weeks after implantation), but no corresponding increase in pecking damage scores was observed. This may suggest a stimulatory effect of oestradiol/progesterone administration on this type of pecking, but such a conclusion should be treated with caution since no sustained effect was observed, and throughout the experiment numbers of vigorous peck/pulls were low and variable. Indeed, at 20 weeks of age, the situation was reversed and numbers of vigorous pecks were higher in control groups. Experiment 1a was characterised by outbreaks of severe aggressive pecking, which were observed after the onset of lay in both treatment and control groups. Such aggressive behaviour was also seen in the experiment described in Chapter 3 (though to a lesser extent). The fact that this behaviour resulted in the deaths of some birds (either directly or by necessitating culls) further demonstrates that aggressive pecking can be a serious welfare problem.

The second hormone administration technique investigated was Silastic implants. In Experiment 2a, implants containing a mixture of oestradiol and progesterone were effective in releasing progesterone, but did not increase plasma oestradiol or plasma triglyceride. Dziuk and Cook (1966) noted that the passage of more polar steroids such as oestradiol through Silastic was poorer than that of less polar steroids such as progesterone, and Lifchez and Scommegna (1970) also noted that progesterone passes through Silastic tubing more easily than other steroids. Administration of oestradiol and progesterone in separate implants in Experiment 2b

produced similar results, with release of progesterone much more effective than oestradiol. Use of thinner walled implants with a larger surface area in Experiment 2b did improve release of oestradiol, but did not achieve circulating oestradiol levels comparable to that of a laying hen or overcome the problem of high variability between treated individuals. Previous studies have described the release pattern from Silastic implants as an initial hormone surge followed by a lower rate of release (Lifchez and Scommegna, 1970; Mashaly et al., 1979), and although this appeared to be the case in the current studies for release of progesterone, the release of oestradiol was variable across time and individuals.

Pullets treated with implants containing the anti-oestrogen tamoxifen exhibited greatly accelerated comb and wattle growth. This apparently profound androgenising effect was due to increased plasma testosterone, which was elevated in a dose dependent manner in response to tamoxifen. A similar effect of tamoxifen on comb growth in pullets attributed to increased androgen production was reported by Jaccoby et al. (1992). As described previously, a negative feedback mechanism exists between gonadal steroids and LH. Blockade of oestradiol receptors at the level of the adeno-hypophysis by tamoxifen prevents the feedback exerted by circulating oestrogen and leads to increased LH secretion. These high circulating levels of LH stimulate gonadal steroidogenic activity thus increasing circulating levels of gonadal steroids (Etches, 1996). Testosterone production appeared to increase to the greatest extent in response to tamoxifen, and this is probably because androgens and oestrogens share a metabolic pathway, with testosterone being a precursor of estradiol, the conversion involving aromatisation (Epple and Stetson, 1980).

No effects on bird to bird pecking, at least in terms of pecking damage scores, were seen in response to increased plasma progesterone levels produced by the implants and the endogenous testosterone increase as a result of treatment with tamoxifen in Experiment 2a. The low levels of damaging pecking seen throughout the experiment may have been due to the small group size (five) and resultant lower stocking density. Hughes (1973) reported an effect of progesterone on the incidence of damaging pecking, and circumstantial evidence for its involvement was provided by the results of the experiment described in Chapter 3. However, the stimulatory

effect on pecking behaviour of progesterone observed by Hughes (1973) occurred to the greatest extent when oestradiol was also administered. This can be explained by the stimulatory effect of oestradiol which 'primes' progesterone receptors (by enhancing their production), which is well known in mammals (Fraile et al., 1987; Bradshaw et al., 1991) and birds (Kawashima et al., 1979; Kamiyoshi et al., 1992; Kawashima et al., 1992). Therefore, the failure of the implants to deliver oestradiol may have reduced any behavioural manifestations of the increased circulating progesterone, because no oestradiol priming of progesterone receptors took place.

Allee et al. (1939) reported increased aggression in testosterone treated hens, and it is interesting to note that no overall increase in levels of aggression were observed in tamoxifen treated birds in Experiment 2a. Although no detailed behavioural observations were carried out, the deleterious results of overt aggression on head and neck plumage and comb condition (as seen in previous studies in this thesis and recently reported by Bilcik and Keeling, 1999) were not apparent. This lack of aggression may be due to the fact that all birds in each group received the same treatment, unlike in the Allee et al. (1939) study, where treated hens gained dominance over untreated hens in staged aggressive encounters.

Experiment 3 tested a novel method of oral steroid administration, where food pellets were coated in wax containing hormone. Although the birds ate the wax after some initial aversion, the oral route of administration was not effective in altering hormonal state. Despite an encouraging plasma triglyceride response to oral oestradiol treatment, which indicated that the hormone was being absorbed, plasma steroid assays provided no evidence that circulating levels of oestradiol or progesterone were altered. Orally administered oestradiol would enter the entero-hepatic circulation, and the increased plasma triglyceride observed in birds receiving the oestradiol diet was probably due to stimulation of lipogenesis in the liver, which is known to be oestradiol mediated (Annison, 1983; Hagan et al., 1984; Hermier et al., 1996). However, the absence of oestradiol (and progesterone) in the plasma indicated that degradation and elimination of the hormones also took place in the liver, preventing them from reaching the general circulation.

It would seem, therefore, that the well-documented poor oral bioavailability of steroids in mammals also applies to birds. Several synthetic hormone alternatives

with improved oral bioavailability have been developed, which are commonly used in oral contraceptives, such as ethinylestradiol (an oestrogen) and norethisterone (a progestogen) (Shenfield and Griffin, 1991). Unfortunately, the quantities of these compounds required to manipulate pullet hormonal state (particularly in a large-scale experiment) would be prohibitively expensive.

In Experiment 4, the effects of the anti-oestrogen tamoxifen on propensity to peck a novel device (a bunch of string) was determined in birds previously identified as peckers and non-peckers. Before treatment, peckers exhibited less gentle pecks and pulls at the device than non-peckers, while no differences between bird types were observed in numbers of vigorous peckers and pulls. These results are not consistent with other studies where a positive association between object pecking and feather pecking has been reported. Channing et al. (1998) found that birds identified as 'peckers' showed greater tendencies to peck a variety of objects, including string bunches, than 'non-peckers', although different types of pecks at the objects were not recorded. A greater tendency to peck 'objects' in a genetic line with a high propensity to feather peck was also reported by Blokhuis and Beuving (1993). It is likely that the type of object, method of presentation, and duration of test all influence measurements of propensity to peck. During testing, the peckers appeared to be more fearful of the device, particularly during its first presentation, and exhibited less gentle, exploratory pecks than non-peckers. An association between damaging pecking and fear has been suggested (Keeling and Jensen, 1995; Jones, 1996), but remains ill defined.

Experiment 4 provided an opportunity to compare the basal hormonal state of adult pecker and non-pecker hens (albeit in small groups). There appeared to be no underlying differences in plasma oestradiol or testosterone concentration, although this does not preclude the possibility of differences between pecking and non-pecking individuals at an earlier stage of development.

Treatment with tamoxifen resulted in physiological changes in both peckers and non-peckers, including reduced plasma triglyceride and elevated plasma oestradiol and testosterone. Similar effects have been observed previously in pullets and hens treated with tamoxifen (Jaccoby et al., 1992; Jaccoby et al., 1995), and reflect increased steroidogenesis in response to elevated gonadotrophin secretion.

Similar effects were seen in Experiment 2a, where juvenile pullets were treated chronically with tamoxifen.

Immediately after treatment, both peckers and non-peckers receiving tamoxifen exhibited a reduced tendency to vigorously peck and pull the presented device, while numbers of gentle pecks and pulls were unaffected. One week later, the situation was reversed and tamoxifen treated birds were vigorously pecking and pulling the string more than controls. These results suggest that some types of pecking behaviour, namely vigorous pecks and pulls, are influenced by oestradiol, and as such were inhibited by tamoxifen. The fact that numbers of gentle pecks and pulls were unchanged reiterates the lack of association between potentially damaging pecks and gentle, non-damaging pecks that has been described by other authors (Savory and Griffiths, 1997; Kjaer and Vestergaard, 1999). The increase in vigorous pecking/pulling in tamoxifen treated birds one week after treatment could be attributed to the elevated circulating steroid levels observed in these birds. Although plasma oestradiol was already elevated in tamoxifen treated hens when the first post-treatment observations took place, it is possible that its effects were inhibited by high circulating tamoxifen levels. Previously in this thesis (Chapter 3) and elsewhere (Hughes, 1973), progesterone has been implicated in the development and control of damaging pecking. Although effects of tamoxifen are likely to be primarily oestradiol receptor mediated, there is some evidence that tamoxifen can regulate progesterone receptors in birds (Sutherland, 1981; Laugier et al. 1991), and as such, a progesterone receptor mediated effect on pecking cannot be ruled out.

This study was carried out on a small number of birds and the apparent suppressive effect of tamoxifen on vigorous pecks and pulls directed at an inanimate object may not be transferable to scenarios involving actual bird to bird pecking. Nevertheless, these results are encouraging and provide further evidence for the involvement of gonadal hormones in the regulation of pecking behaviour.

In conclusion, the lack of meaningful results here were due to a combination of failure of various attempts to alter hormonal state by administration of exogenous gonadal steroids and, in one experiment, a complete absence of damaging pecking. However, evidence that gonadal hormones were causally associated with vigorous

pecking/pulling at a pecking device was obtained, when numbers of pecks at string were suppressed in tamoxifen treated hens.

Chapter 5

Is development of pecking damage in pullets influenced by dietary protein source?

5.1 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), restrictions imposed by some retailers for free range eggs, 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 levels of pecking damage in laying hens. There have been anecdotal reports of outbreaks of feather pecking and cannibalism after changes in the diet from mainly animal to mainly plant protein (e.g. Curtis and Marsh, 1992), and it is the opinion of some egg producers that the presence of animal protein in layer diets (especially fishmeal) is effective in preventing or even halting outbreaks of damaging pecking. In support of the notion that animal protein can suppress damaging pecking, the Farm Animal Welfare Council stated in its 1997 Report on the Welfare of Laying Hens, “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 due to cannibalism (Cain et al., 1984; Ambrosen and Petersen, 1997), but the question of protein source effects on the incidence of pecking damage has not been addressed. Some animal/plant protein source comparisons have been carried out, but only examining effects on performance traits such as egg production (Mundheim and Opstvedt, 1981, Al Bustany and Elwinger, 1987a) and egg quality (Al Bustany and Elwinger, 1987b). 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 to only bloodmeal or soybean meal. In the only study to directly examine effects of dietary protein source on pecking damage, Savory et al. (1999) compared levels of pecking damage in growing bantams (0-6 weeks of age) fed isonitrogenous diets based on either plant (soyabean meal), animal (bloodmeal, fishmeal, hydrolysed feather meal) or semipurified (casein) protein. No difference in pecking damage scores between treatments was observed. In fact, an effect of dietary protein source on the incidence of pecking damage in adult or juvenile commercial layers has never been demonstrated, and no information is currently available on which FAWC could have based their recommendations (above).

It has been assumed that the alleged suppressive effects on pecking induced by fishmeal are due to a nutrient found only in animal protein sources, such as the 'animal protein factor' vitamin B₁₂ (Bolton and Blair, 1974). However, it is also conceivable that any effects on pecking behaviour could be due to detrimental substances present in plant protein sources. Phytoestrogens are plant compounds which resemble steroidal oestrogens in molecular structure and can behave agonistically or antagonistically, depending on the species. In humans, many phytoestrogens act as oestradiol antagonists, as indicated by epidemiological studies demonstrating that the risk of certain diseases such as breast cancer are lower for women in certain geographical areas consuming diets high in phytoestrogens (Knight and Eden, 1995; Bingam et al., 1998). Conversely, phytoestrogens have been shown to act agonistically in fish, inducing vitellogenesis (as oestrogen does) in cultured Siberian Sturgeon (Pelissero et al., 1991). Phytoestrogens are found in many plant species, some of which are used as feedstuffs for poultry (Sheehan, 1993; 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 (100%). Despite these relatively low potencies, phytoestrogens are present in high concentrations in some feedstuffs (1800mg/kg isoflavones in soyabeans, for example (Bingham et al., 1998)) and are therefore potentially biologically important.

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 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). Most importantly, elevated plasma oestradiol levels 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 oestradiol levels 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). Previous findings in this thesis implicate gonadal hormones, in particular oestradiol, in the development of pecking damage (Chapter 4), and a demonstrated association exists between oestradiol and activity level (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). Hughes (1973) demonstrated increased damaging pecking in juvenile pullets treated chronically with a combination of oestradiol and progesterone. It is possible, therefore, that oestrogen-like compounds in plant protein sources could affect pecking behaviour. If this is the case, then effects of phytoestrogens could depend on 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). Effects of dietary protein source on behaviour, growth, egg production and hormonal state were determined in juveniles and through sexual maturity into the laying period,

using the same experimental approach (0-24 weeks of age) as the behavioural development experiment described in Chapter 3. Because of previous unpredictability observed in pecking behaviour, the experimental design was simple, two dietary treatments with six groups in each.

An additional aim of this experiment was to relate individual variation in pecking behaviour to hormonal state. No previous studies have compared physiological parameters in high and low pecking individuals, although low plasma corticosterone and elevated heart rate during manual restraint in a 'high' feather pecking genetic line compared to a 'low' pecking line has been demonstrated (Korte et al., 1997; Korte et al., 1999). The methodology of previous studies in this thesis (blood sampling a subset of birds per group) precluded this approach. Although no basal differences were observed between the eight 'pecker' and eight 'non-pecker' hens used in Chapter 3 (Experiment 4), differences may not have been apparent in such a small groups of birds. Outbreaks of damaging pecking took place in several groups during this experiment, so the opportunity was taken to blood sample all 104 birds left at the end of the experiment (25 weeks), in an attempt to relate plasma parameters to pecking behaviour.

5.2 Methods

One hundred and forty-four ISA Brown pullets were randomly allocated at day old into 12 groups of 12 and reared in pens to 24 weeks of age as described in Section 2.2. At day-old, groups were randomly assigned to "animal" or "plant" protein treatments, 6 groups per treatment, and as such were fed starter (0-6 weeks), grower (7-16 weeks) and layer (17-24 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 5.1), full formulations are shown in Table 5.2. The crude protein/metabolisable energy ratios for the animal and plant diets were 15.1 and 15.0g/MJ for the starter diets, 12.5 and 12.5g/MJ for the grower diets, and 13.7 and 13.9g/MJ for the layer diets. Vitamins and minerals were equalised and special care was taken to equalise the amino acid tryptophan, which has been shown to influence pecking behaviour previously (Savory et al., 1999). All diets were produced at Roslin Institute 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) which is obtained by 'drying and grinding whole fish or parts thereof of various species'. Soyabean meal (48% protein) and rapeseed meal were included in the plant protein diet, since rapeseed meal is increasingly included in commercial plant protein diets as improved cultivars become available (Hulan and Proudfoot, 1981).

		CP (g/kg)	TME (MJ/kg)	calcium (g/kg)
Starter (0-6wks)	Animal	178	11.8	9.5
	Plant	180	12.0	9.0
Grower (7-16wks)	Animal	149	12.0	8.1
	Plant	150	12.0	8.0
Layer (17-24 wks)	Animal	161	11.8	32.6
	Plant	163	11.7	32.4

Table 5.1 Calculated values for crude protein (CP), energy (TME) and calcium in the animal and plant protein starter, grower and layer diets

<i>Dietary constituent (g/kg)</i>	Starter		Grower		Layer	
	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	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

Table 5.2 Complete formulations of the animal (A) and plant (P) protein diets.

Regular measurements of growth, behaviour (ethogram and pecking counts, see Section 2.4), pecking damage scores, egg production and blood sampling were carried out as described in Chapter 2, according to the schedule shown in Table 5.3. Collection of blood samples and measurement of comb and wattle growth were carried on a subset of three birds (leg band colours blue, red, and green, assigned at random) in each pen in alternate weeks from 10 weeks of age. Blood samples were assayed for plasma zinc and triglyceride, and a limited number of samples were assayed for oestradiol and progesterone (as described in Chapter 2). An additional blood sample was obtained from every bird remaining in the experiment at 25 weeks (104 birds), these ‘survey’ samples were assayed for oestradiol, progesterone and testosterone (as described in Chapter 2).

<i>Week</i>	1	2	3	4	5	6	7	8	9	10	11	12
Body weight		✓		✓		✓		✓		✓		✓
Comb and wattle growth*										✓		✓
Ethogram		✓		✓		✓		✓		✓		✓
Bird to bird pecking	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Pecking damage scores		✓		✓		✓		✓		✓		✓
Egg Production												
Blood Sampling*										✓		✓

<i>Week</i>	13	14	15	16	17	18	19	20	21	22	23	24
Body weight		✓		✓		✓		✓		✓		✓
Comb and wattle growth*		✓		✓		✓		✓		✓		✓
Ethogram		✓		✓		✓		✓		✓		
Bird to bird pecking	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
Pecking damage scores		✓		✓		✓		✓		✓		✓
Egg Production				✓	✓	✓	✓	✓	✓	✓	✓	✓
Blood Sampling*		✓		✓		✓		✓		✓		✓

Table 5.3 Schedule of measurements in the dietary protein experiment, those marked with * were carried out on the same subset of three birds per group.

Student's t-test comparisons were carried out to determine treatment effects at each age for bodyweight, comb and wattle growth, egg production and plasma parameters. Data from behaviour observations were summarised into age ranges (weeks 2 and 4, weeks 6, 8 and 10, weeks 12,14 and 16 and weeks 18, 20 and 22 for ethogram behaviours, and weeks 1-4, 5-8, 9-12, 13-16, 17-20 and 21-24 for pecking counts), and the treatments compared by t-tests for each age range. A correlation matrix was constructed for each pen to investigate relationships between pecking behaviour (total numbers of vigorous pecks, feather pulls and aggressive pecks given by each bird between weeks 0-16 and 17-24, log transformed) and plasma hormones in the 'survey' blood samples taken at 25 weeks.

5.3 Results

5.3.1 Bodyweight

The growth curve exhibited by the pullets was similar to that seen in previous experiments (see Section 3.3.1). Dietary treatment had a significant effect on bodyweight, with groups receiving the animal protein diet being heavier than those fed the plant protein diet (Figure 5.1). Although the bodyweight difference between treatments was small, it was consistent and significant at every age from 4 weeks onwards (t-tests with pen means, $P < 0.05$, $n = 12$). Within treatments, variation in bodyweight between birds was small (mean CV across ages for animal and plant protein of 0.04 and 0.03, respectively).

5.3.2 Comb and wattle growth

Rapid comb and wattle growth was observed in both treatment groups from 14 weeks of age onwards (Figure 5.2). Animal protein groups had larger combs and wattles than plant protein groups throughout the experiment; combs were significantly larger in animal protein groups at 10, 12 and 14 weeks (t-test, $P < 0.01$, $P < 0.05$, $P < 0.01$, respectively, $n = 12$). Wattles were significantly larger in animal protein groups at 20 weeks of age (t-test, $P < 0.05$, $n = 12$).

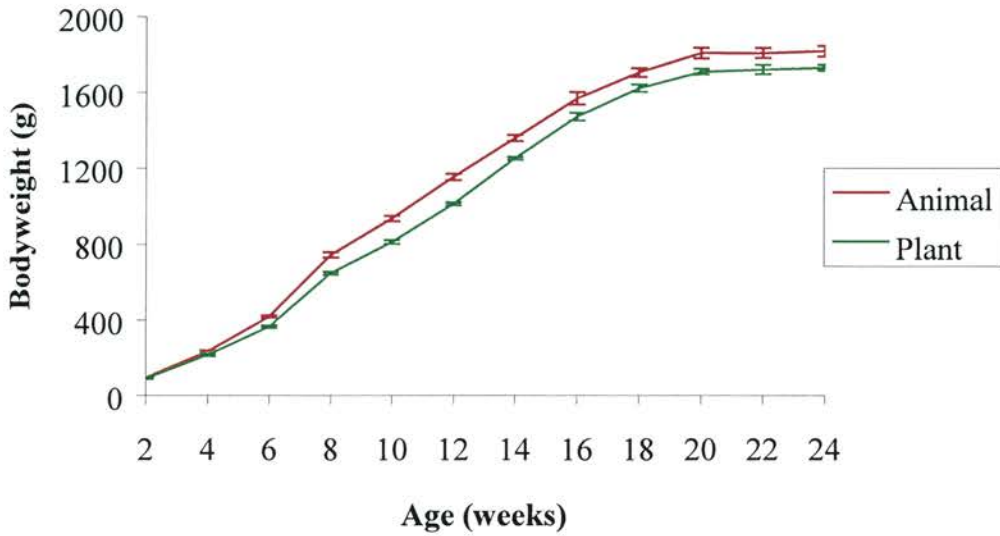


Figure 5.1 Mean bodyweights (\pm SE) from 2 to 26 weeks in groups of 12 pullets receiving animal or plant protein based diets (6 groups per treatment).

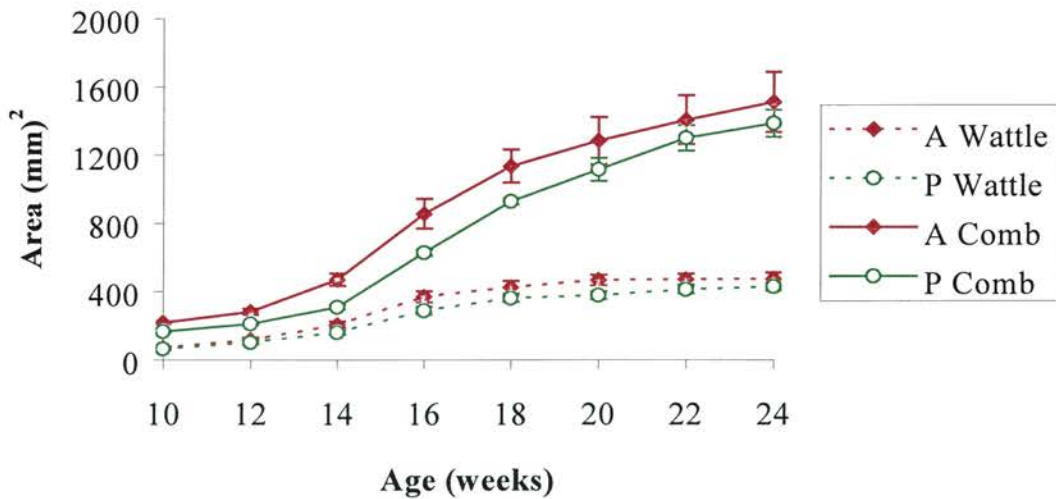


Figure 5.2 Mean comb and wattle growth (\pm SE) between 10 and 26 weeks in groups of 12 pullets receiving diets based on animal (A) or plant (P) protein (6 groups per treatment).

5.3.3 Ethogram

Age and treatment effects were observed in the proportion of time spent in ethogram behaviours (Figure 5.3). As reported previously (Section 3.3.3), standing, walking and litter directed behaviour took up a large portion of the time budget. Students t-test comparisons of animal and plant protein groups at each age range yielded four significant results. Animal protein groups spent significantly more time standing than those receiving the plant protein diet, between 0-4 weeks ($P < 0.01$, $n = 12$) and 6-10 weeks ($P < 0.05$). Between 0-4 weeks, plant protein groups spent more time in litter directed behaviour than animal protein groups ($P < 0.05$, $n = 12$). Between 6-10 weeks, plant protein birds spent more time pecking other birds (aggressively or non-aggressively) than those on the animal protein diet ($P < 0.05$, $n = 12$). No significant differences were observed between treatments in age ranges 12-16 weeks and 18-22 weeks.

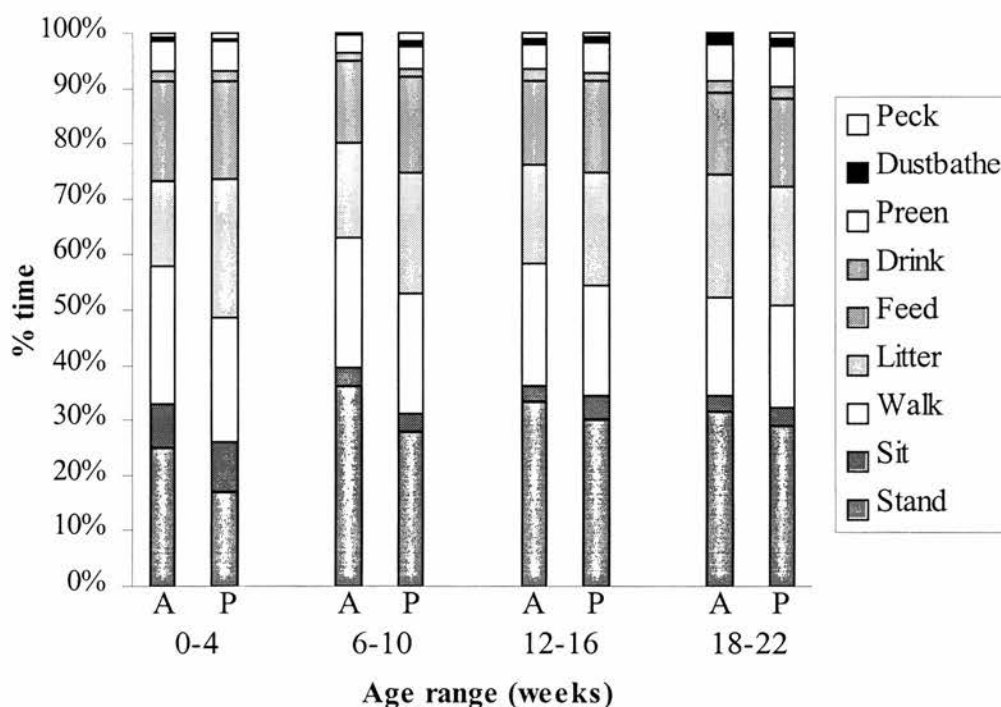


Figure 5.3 Mean time spent in ethogram behaviours between 0 and 22 weeks in groups of pullets receiving diets based on animal (A) or plant (P) protein (6 groups per treatment).

5.3.4 *Bird to bird pecking*

There were no significant differences between treatments in numbers of gentle pecks given/received any age range, and an increase was seen in both treatment groups at the onset of lay (17-20 weeks, Figure 5.4). A consistently higher number of vigorous pecks/pulls were seen in plant protein groups throughout the experiment (Figure 5.4), but the number was significantly higher in only one age range, 13-16 weeks ($P < 0.05$, t-test, $n=12$). Numbers of aggressive pecks increased after sexual maturity (Figure 5.6), and were not significantly affected by dietary treatment.

5.3.5 *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 (Section 5.3.4). Pecking damage was first seen at six weeks of age (Figure 5.7), and 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 scores were minimal. After a peak at 10 weeks, pecking subsided and damage scores fell before rising again at 16 weeks. Cannibalism first occurred at 18 weeks of age, and 13 deaths (7 of these were culls) occurred due to cannibalism (predominantly vent pecking) during the experimental period (see Table 5.4 for mortality summary). While pecking damage scores were recorded in all groups, severe pecking damage took place in only four, Pen 2 (plant), Pen 5 (plant), Pen 7 (animal) and Pen 12 (animal), and three of these (pens 2, 5 and 7) were removed from the experiment prematurely (see Section 2.5 for group removal criteria).

As observed previously (Section 3.3.5) some birds were killed or had to be removed as a result of aggressive pecking (Table 5.4), although the extent of this problem was less than that observed in Chapter 3. The pens exhibiting severe aggression were Pen 5 (plant), Pen 7 (animal) and Pen 10 (animal), and a total of five deaths occurred (four of these were culls).

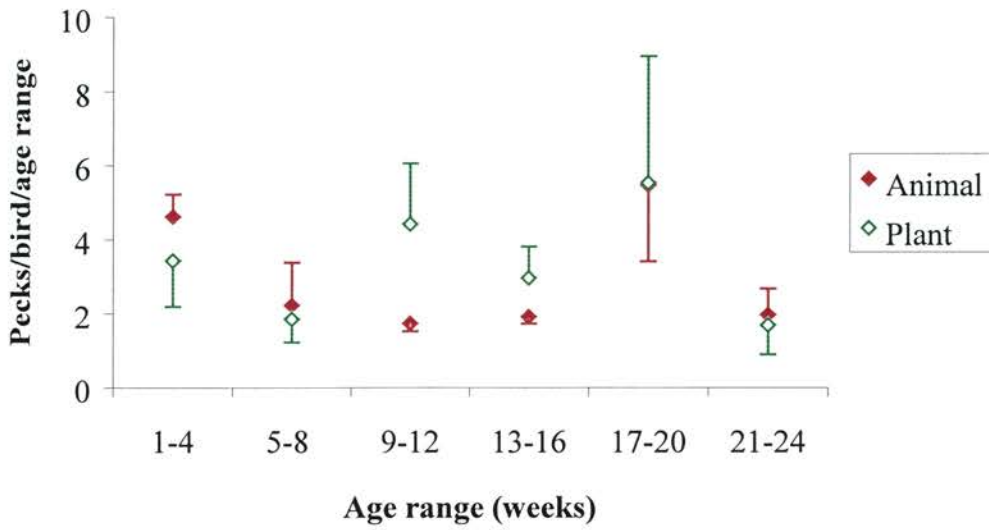


Figure 5.4 Age related changes (across 6 age ranges) in mean numbers of observed gentle pecks (\pm SE) in groups of pullets receiving diets based on either animal or plant protein (6 groups per treatment).

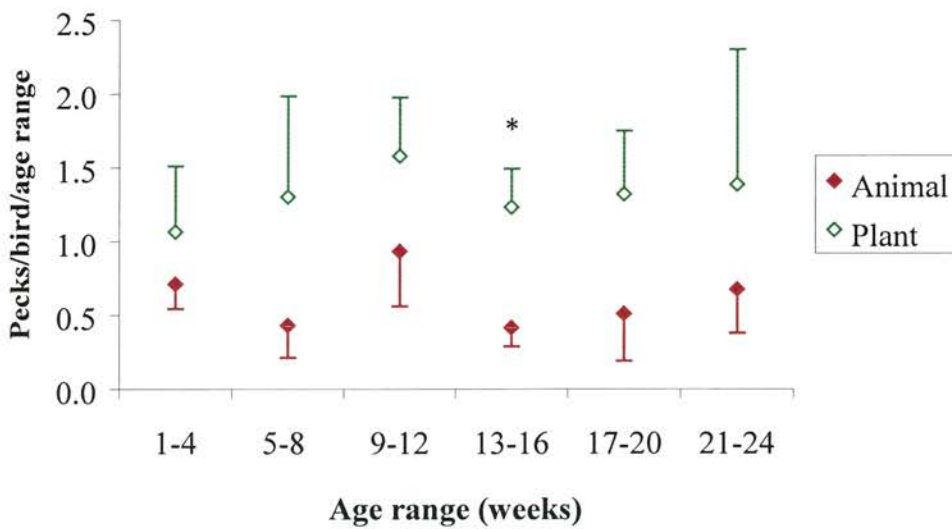


Figure 5.5 Age related changes (across 6 age ranges) in mean numbers of observed vigorous pecks/pulls (\pm SE) in groups of pullets receiving diets based on either animal or plant protein (6 groups per treatment). * $P < 0.05$

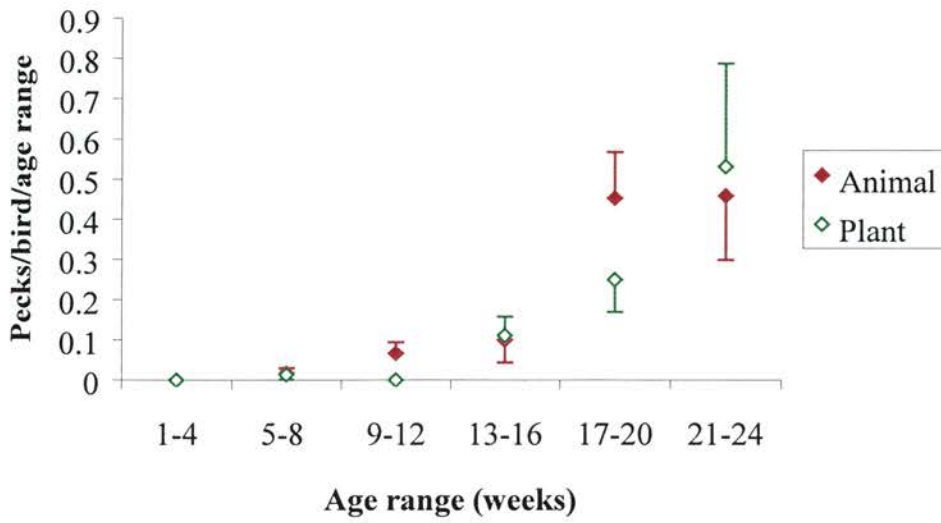


Figure 5.6 Age related changes (across 6 age ranges) in mean numbers of observed aggressive pecks (\pm SE) in groups of pullets receiving diets based on either animal or plant protein (6 groups per treatment).

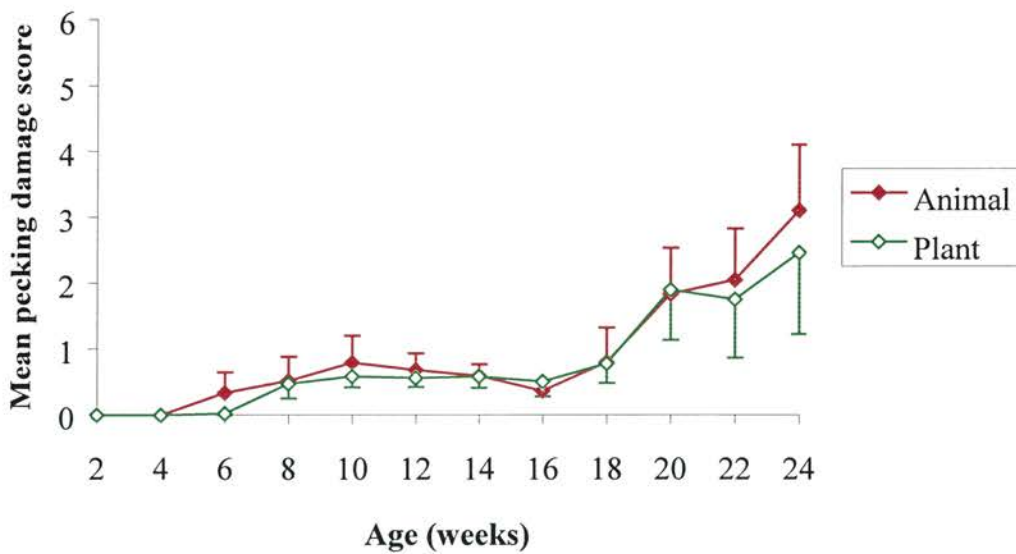


Figure 5.7 Mean pecking damage scores (\pm SE) between 2 and 24 weeks of age in groups of pullets receiving diets based on either animal or plant protein (6 groups per treatment).

		Age (weeks)								
	Diet	16	17	18	19	20	21	22	23	24
Pen 1	A									
Pen 2	P						●	●●●	—	—
Pen 3	A									
Pen 4	P									
Pen 5	P			●	○		●●	—	—	—
Pen 6	P									
Pen 7	A			○				●		●●
Pen 8	A									
Pen 9	P									
Pen 10	A					○○				
Pen 11	P									
Pen 12	A									

● Cannibalism ○ Aggression
 — group removed from experiment (see Section 2.5)

Table 5.4 Number and types of mortality per pen between 16 and 24 weeks in pullets fed animal (A) or plant (P) protein based diets. Each dot represents one bird.

5.3.6 Egg production

Egg production commenced at 16 weeks of age, and all groups were in lay by 17 weeks of age (Figure 5.8). Egg production rose steadily, approaching 0.8 eggs hen/d by the end of the experiment at 24 weeks. Numbers of normal or abnormal eggs were not affected by dietary treatment at any age. Mean egg weights at 26 weeks were $53.7 \pm 0.28\text{g}$ (animal) and $54.1 \pm 0.17\text{g}$ (plant); these did not differ between treatments at any age.

5.3.7 *Plasma assays*

Dietary treatment had no effect on plasma triglyceride or plasma zinc concentrations, which both showed rapid increases between 16 and 18 weeks (reflecting the onset of lay) and remained elevated (Figure 5.9). Mean plasma triglyceride concentration increased from less than 2mg/ml to over 10mg/ml. Mean plasma zinc concentrations increased from 2-3ug/ml to over 5ug/ml.

Plasma oestradiol profiles were similar to previous measurements at these ages (Section 3.3.7), with a prepubertal peak (levels increasing from between 100-200 pg/ml to over 300 pg/ml) apparent in both treatment groups (Figure 5.10). Plasma progesterone increased rapidly between 16 and 20 weeks of age (Figure 5.11) in both treatment groups, as observed previously (Section 3.3.7). No significant effects of dietary treatment on plasma oestradiol or progesterone were detected at any age.

5.3.8 *Survey samples*

There were no significant correlations within pens between plasma hormone levels at 25 weeks and vigorous pecks, feather pulls or aggressive pecks either before (0-16 weeks) or after (17-24 weeks) the onset of lay. For pecking counts alone, four significant correlations were apparent. In Pen 4, the same individuals delivered feather pulls before and after the onset of lay ($P < 0.01$), and the same individuals were delivering both vigorous pecks and feather pulls ($P < 0.01$) before the onset of lay. In Pen 10, individuals delivering more feather pulls as juveniles delivered more vigorous pecks after the onset of lay ($P < 0.05$), and in Pen 3 before the onset of lay, the same individuals were delivering both vigorous pecks and feather pulls ($P < 0.01$).

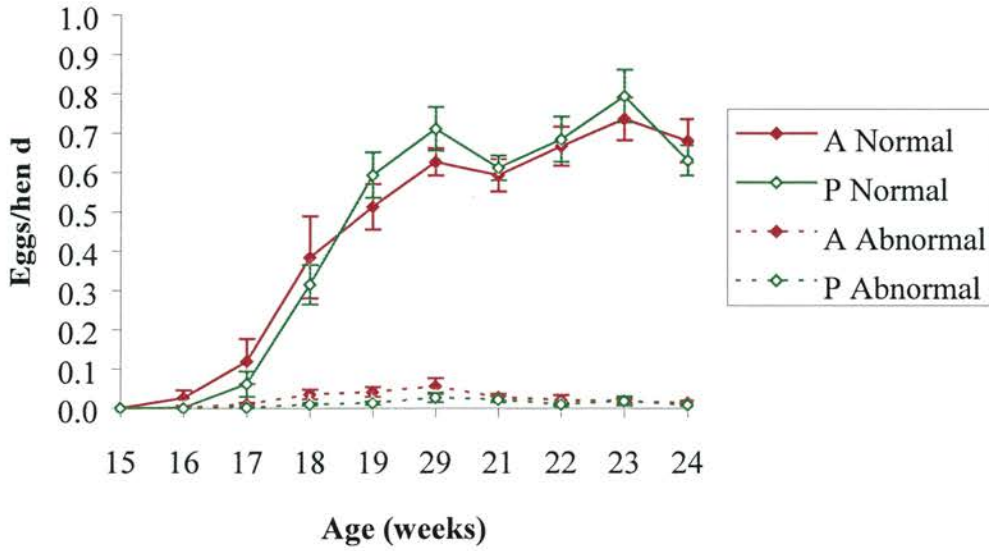


Figure 5.8 Mean normal and abnormal egg production in eggs/hen d (\pm SE) between 15 and 24 weeks of age in groups of pullets receiving diets based on animal (A) or plant (P) protein (6 groups per treatment).

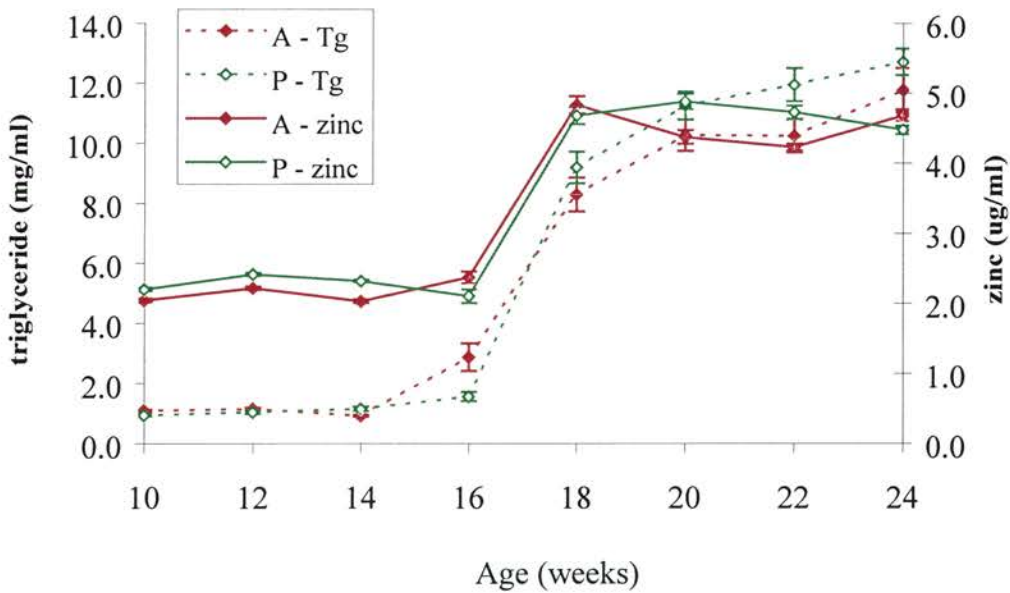


Figure 5.9 Mean plasma triglyceride (Tg) and zinc concentrations (\pm SE) between 10 and 24 weeks of age in pullets receiving diets based on animal or plant protein (n=18 per treatment at each age).

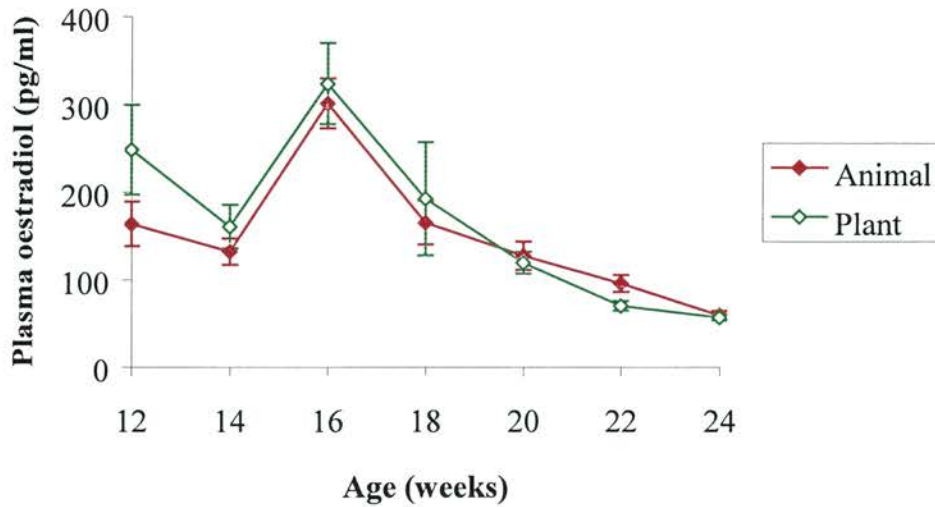


Figure 5.10 Mean plasma oestradiol concentration (\pm SE) between 12 and 24 weeks of age in pullets receiving diets based on animal or plant protein (n=12 per treatment at each age).

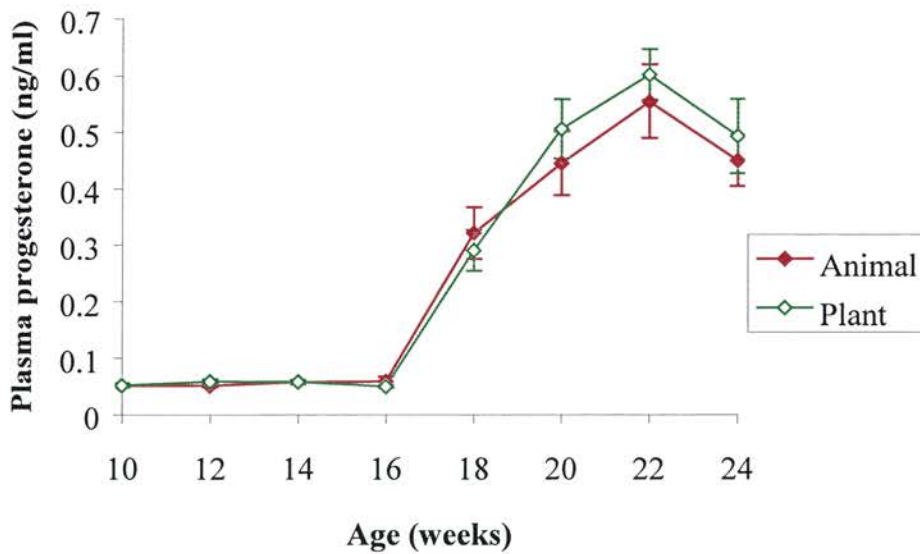


Figure 5.11 Mean plasma progesterone concentration (\pm SE) between 12 and 24 weeks of age in pullets receiving diets based on animal or plant protein (n=12 per treatment at each age).

5.4 Discussion

Consistently higher bodyweights were observed in pullets receiving the animal protein diet. Although attempts were made to measure feed 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, 1987a). Since 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.

Larger comb and wattle size in the animal protein groups was probably related to the bodyweight difference, but may also reflect a difference in levels of circulating androgen, which promotes comb growth (Itoh et al., 1988; Jacoby et al., 1992). Plasma testosterone was measured at 25 weeks, and was higher in animal protein birds.

Differences between the animal and plant protein groups in time spent in ethogram behaviours were apparent between 0-4 weeks (standing and litter directed behaviour) and 6-10 weeks (standing and bird to bird pecking). Less time spent standing (doing nothing else) and more time spent pecking and scratching litter could indicate higher activity levels in plant protein groups, although no difference was observed between treatments in time spent sitting (which is often used as a measure of inactivity). Given the demonstrated association between oestradiol and activity level in various species including the domestic fowl (Horne and Wood-Gush, 1970; Cushing et al. 1995), these results could suggest an oestrogenic effect of the plant protein diet. This cannot be confirmed since measurements of plasma oestradiol did not take place before 10 weeks of age; however, there was no evidence of an influence of dietary protein source on circulating hormone levels after 10 weeks (see later discussion). Increased litter directed activity in juveniles has been associated with reduced feather pecking in later life (Blokhuys and van der Haar, 1989; Blokhuys

and Beuving, 1993; Blokhuis and Wiepkema, 1998). However, there was some evidence (counts of vigorous pecks/pulls) that damaging pecking was greater in plant protein groups (which exhibited more litter directed activity) 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 6-10 weeks of age. The reason for this anomaly is not clear, but it is possible that increased general activity may have manifested itself as both litter directed activity and bird to bird pecking. In the experiment described in Chapter 3, no difference in litter directed activity was detected between high and low pecking groups.

Detailed observations of pecking behaviour revealed no treatment effects on numbers of gentle, non-damaging feather pecks, with a large increase in gentle pecks seen in both treatments at the onset of lay (17-20 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). In contrast, consistently higher numbers of vigorous, potentially damaging pecks and pulls were observed in plant protein groups throughout the experiment (significant only in one age-range, 13-16 weeks). The results of pecking observations, therefore, tend to support the notion that damaging pecking is promoted by plant protein based diets.

Surprisingly, no differences were observed between treatments in pecking damage scores, which did not reflect the increased vigorous pecking/pulling observed in plant protein groups. Pecking damage was first seen at 6 weeks, peaked temporarily at 10 weeks, and then increased again at 16 weeks (onset of lay), after which deaths due to cannibalism (vent pecking) occurred in animal and plant protein groups. Interestingly, the age related changes in numbers of vigorous pecks/pulls reflect those seen in pecking damage scores – a peak between 9-12 weeks, a fall and then an increase from 17 weeks onwards.

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

Dietary protein source had no effect on the indirect plasma indices of reproductive state, zinc and triglyceride (see Section 2.8). Measurements of plasma oestradiol and progesterone, which did not significantly differ between treatments, confirmed that dietary protein source had no influence on hormonal state. These results are in contrast to previous reports of elevated plasma oestrogen and lipid in laying hens fed corn-soy 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 corn/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 levels in the grower and layer diets may account for the lack of oestradiol/lipid response. Levels 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, 1976). It is possible that one or all of these factors influenced the levels 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. The inclusion of rapeseed meal in the plant protein diets (5, 4 and 5% in the starter, grower and layer diets), which has a low phytoestrogen content, may have had a diluting effect on the soybean.

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 levels of oestradiol, oestrone and androgens in Norwegian fishmeal (similar to that used in this experiment) and noted wide batch variation in steroid content. Although steroids in fishmeal are present at lower concentrations than phytoestrogens in soybean meal (ng/100g compared to mg/100g), their much 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 levels of both progesterone and oestradiol were within ranges expected for age.

The 'survey' samples taken at 25 weeks of age were an attempt to relate individual variation in pecking behaviour to hormonal state. There is a paucity of information regarding the physiological characteristics of high and low pecking individuals, with the only available studies comparing genetic lines with different propensities to feather peck. Individuals from a high pecking line have been shown to have higher heart rates (Korte et al., 1999) and lower corticosterone levels (Korte et al., 1997) during manual restraint than those from low pecking lines. In the present study, no significant correlations were apparent between feather pecks or pulls delivered and plasma concentrations of oestradiol, progesterone or testosterone. There are several possible reasons why no associations were observed. Firstly, plasma oestradiol, progesterone and testosterone are all subject to fluctuations associated with the ovulatory cycle (Senior, 1974b; Etches and Cunningham, 1976; Etches and Cunningham 1977). Despite taking care to sample birds at a time of day (afternoon) when such effects were likely to be minimal (particularly avoiding pre-ovulatory peaks), nevertheless, they probably represent a source of variation in the single point measurements examined. Secondly, it is not possible to determine to what extent plasma hormone levels at 25 weeks were representative of those earlier in the experiment, and it is possible that the strength or position of correlations may have been different at other stages of development. Finally, by 25 weeks, three groups where severe pecking had occurred had been removed, which may have affected the results.

Associations were apparent in some pens for individual pecking behaviour before and after the onset of lay. Between 0-16 weeks, individuals' rates of vigorous pecking and pulling were associated, agreeing with previous studies suggesting that some individuals specialise in these behaviours (Savory and Griffiths, 1997; Wechsler et al., 1998). The results also suggest that the same individuals were delivering feather pulls before and after the onset of lay, which indicates that the same birds are responsible for damaging pecking during different phases of development.

In conclusion, these results do not provide convincing evidence to support the notion that the presence of fishmeal in layer diets suppresses pecking damage, since feather pecking and cannibalism occurred in both animal and plant protein treatment

groups. However, there was a tendency for higher numbers of vigorous, potentially damaging pecks/pulls in the plant protein groups throughout the experiment. The lack 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. The results of this study suggest a possible effect of dietary protein source on the development of pecking damage which merits further investigation.

Chapter 6

Feather eating and its possible role in the development of pecking damage

6.1 Introduction

Feather eating has been observed in a few species of birds, namely parrots (Hollmann, 1997), grebes (Simmons, 1956; Piersma & van Eerden, 1989) and both commercial layer (Savory & Mann, 1997) and bantam (Savory & Griffiths, 1997; Savory & Mann, 1999) strains of domestic fowl. Feather eating in great crested grebes has been studied in detail where the behaviour is known to have a specialized function relating to feeding. The ingested feathers add bulk to the stomach contents, enabling indigestible remains of the fish diet to be ejected as pellets (Piersma & van Eerden, 1989).

The function of feather eating in domestic fowls is unclear, since this granivorous species does not possess the ability to break down keratin in the digestive tract and feathers cannot, therefore, have any nutritive value. This suggests that feather eating in the domestic fowl is a form of pica (consumption of non-nutritive material with no apparent function). Reports of pica in birds are generally anecdotal and have concentrated on inappropriate ingestion of materials with harmful consequences, for example in ostriches (Stewart, 1994; Samson, 1996) and broiler type chickens (Hutson, 1978). It was recently reported that laying hen chicks and adults readily ingested polystyrene offered as a foraging substrate (Huber-Eicher and Wechsler, 1998; Wechsler and Huber-Eicher, 1998). It is possible that the likelihood of pica may be increased for species (such as the domestic fowl) in which the ingestion of grit to aid digestion is adaptive.

Few previous studies describe feather eating in the fowl, although early workers observed that 'chickens with the feather pulling vice' regularly ate the feathers they removed (Willimon and Morgan, 1953). More recently, results suggesting a possible role for feather eating in the development of feather pecking have been reported,

although no definite link was shown. Savory and Mann (1997) found strain-related variation in sample counts of moulted feathers on pen floors, and observed ingestion of feathers pulled from pecked birds and, more rarely, eating of moulted feathers from the litter floor. Savory and Mann (1999) attempted to quantify the extent of observed feather eating in bantams by examining faecal droppings for evidence of (undigested) feather material. Between 2-15% of droppings were found to contain feathers, but these proportions were not correlated with pecking damage.

This chapter brings together information relevant to feather eating from experiments described in Chapters 3, 4 and 5. Interest in feather eating began during the experiment described in Chapter 3, and thereafter, measurements of feather eating were made in the other major experiments. Approaches used previously by Savory and Mann (floor feather counts and examination of droppings) were employed, as well as direct observation of feather eating and pecking behaviour. Experiment 1 was designed to estimate the extent of feather eating in juvenile birds, while Experiment 2 was designed to give more comprehensive information about age-related changes in feather eating behaviour. Experiment 3 investigated to what extent feather eating in juveniles was a predictor of damaging pecking in adulthood. Experiment 4a directly examined individual variation in propensity to feather peck and feather eat in known 'peckers' and 'non-peckers'. The same birds were used in Experiment 4b to investigate the possibility that the presence of preen oil may contribute to the attractiveness of feathers to eat. The overall aim was to quantify the extent of feather eating behaviour in both juvenile and adult commercial layer pullets, and investigate fully any possible relationship between feather eating and damaging pecking.

6.2 Experiment 1 - Feather eating and damaging pecking in juvenile pullets

6.2.1 Introduction

During the experiment described in Chapter 3, designed to investigate behavioural development in pullets, it was noticed that there was wide variation between groups in the availability of moulted feathers on the floor. The extent of feather eating in the juvenile pullets was therefore examined, and information from behaviour observations and plumage damage scores, carried out as part of the larger study, allowed

an investigation of the relationship between feather eating and feather pecking.

6.2.2 *Methods*

This experiment was carried out concomitantly with the study described in Chapter 3, and therefore the same 144 ISA Brown layer pullets were used. A description of housing and husbandry is given in Chapter 3 (Section 3.2).

At 12 weeks of age the availability of loose feathers on the floor of each pen was estimated. All feathers within two 0.5 x 0.5m quadrats placed at random on the pen floor were collected, counted and measured to the nearest cm. An overall frequency distribution of feather lengths (totals across pens) was produced. The bimodal distribution of feather lengths (Figure 6.1) allowed the categorisation of feathers as 'short' (up to 10cm) or 'long' (>10cm). It was also noted whether feathers were 'downy' (>75% of length downy) and whether they had been partially eaten. This was indicated by a particular type of damage to feathers, with larger primary feathers being most affected. Damage resulting from feather eating was usually seen as one or more notches (or in some cases large areas) missing from the vane of the feather, with the edges around the missing area appearing rough and uneven (Plate 6.1). Feathers were returned to the appropriate pen after counting and measurement.

At 14 weeks of age, 100 faecal droppings of approximately equal size were collected from the floor of each pen (many of these had been trodden on and flattened, and may have been incomplete). The droppings were broken and examined for evidence of feather material (which is not digested and can be clearly seen within droppings). Numbers of droppings containing feather material were recorded, no attempt was made to quantify the feather material.

Data from behaviour observations of bird to bird pecking and pecking damage scores collected between 5-14 weeks of age as part of the larger behavioural development experiment were utilised. Total counts of aggressive pecks, non-aggressive pecks, feather eating events and mean pecking damage scores between 5-14 weeks were calculated for each group. A Spearman rank correlation matrix for the seven variables measured was carried out.



Plate 6.1 Moulted primary feathers from ISA Brown pullets; complete (left) and partially eaten (right).

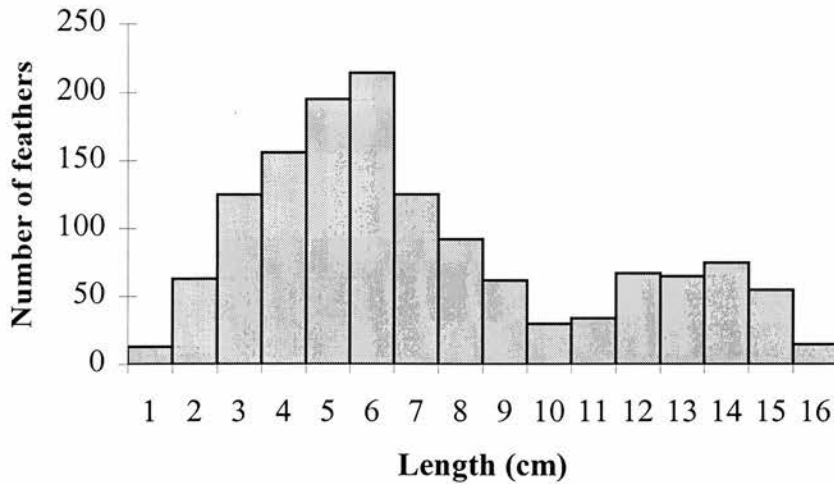


Figure 6.1 Overall frequency distribution of feather lengths from loose feathers collected from all pens at 12 weeks of age in Experiment 1.

6.2.3 Results

There was much variation between pens in total numbers of non-aggressive pecks observed (Table 6.1), ranging from 36 to 173 (overall mean 91.4). Few aggressive pecks were observed, ranging from 0 to 19 (overall mean 3.8), accounting for only a small proportion of total pecks (3.9% across all pens).

Pecking damage occurred in 5 of the 12 pens, and was greatest in Pen 1, less severe in Pens 6, 7 and 10, and minimal in Pen 8. Overall, the tail was most affected by pecking damage, accounting for 89% of total scores. There was no mortality due to pecking damage in juveniles (see Section 3.3.5 for more details).

Feathers were rarely seen being eaten, and numbers of observed feather eating events ranged from 0 to 17 (overall mean 6.4). However, feather material was found in faecal droppings collected from all 12 pens sampled at 14 weeks of age, at levels between 7 and 48% (overall mean 23.7%) (Table 6.1).

Numbers of short feathers on the floor, sampled at 12 weeks, varied much more between pens (Coefficient of Variation, $CV = 0.97$) than did numbers of long feathers ($CV = 0.37$). Thus, the overall variation between pens in numbers of loose feathers was mainly due to variation in the number of short feathers available.

	Non-aggressive pecks/pulls	Aggressive pecks	Feather eating events	Pecking damage scores	% Droppings containing feathers	Short feathers	Long feathers
Pen 1	131	19	9	11.7	41	13	22
Pen 2	57	2	11	0	31	31	12
Pen 3	60	2	5	0	16	105	43
Pen 4	57	0	4	0	9	237	34
Pen 5	98	1	8	0	12	192	52
Pen 6	173	7	5	4.1	48	2	26
Pen 7	36	5	17	1.9	22	26	35
Pen 8	133	3	7	0.3	15	92	21
Pen 9	44	2	0	0	19	122	34
Pen 10	75	3	2	1.3	29	3	23
Pen 11	82	1	2	0	7	192	29
Pen 12	151	0	7	0	35	23	21

Table 6.1 Numbers of pecks, feather eating events and mean damage scores from 5-14 weeks, and feather material in droppings (14 weeks) and floor feather counts (12 weeks) in Experiment 1.

Cross correlations (Spearman rank) among the 7 variables measured (Table 6.1) produced 6 significant results (Table 6.2). Numbers of short feathers on the floor were highly correlated, negatively, with percentages of droppings containing feathers. No such correlation was found with long feathers. Mean pecking damage scores were correlated negatively with numbers of short feathers on the floor, and positively with percentages of droppings containing feathers. Numbers of aggressive pecks were correlated positively with pecking damage scores and proportions of droppings containing feathers, and negatively with numbers of short feathers on the floor.

During behaviour observations, feather eating was seen in six situations. Eating feathers from the pen floor (litter) was most common, accounting for 38% of the 77 observed feather eating events. 34% of feathers eaten were caught in the air, and 19% were removed from other birds. The drinker, feeder, self (while preening) and stealing from other birds were the rarest sources of eaten feathers accounting for between 1% and 5% of the total.

r_s values	Non-aggressive	Aggressive	Feather eat	Peck damage	Droppings	Short
Aggressive	0.115					
Feather eat	0.035	0.292				
Peck damage	0.289	0.904***	0.298			
Droppings	0.333	0.573*	0.337	0.585*		
Short	-0.411	-0.695*	-0.255	-0.750**	-0.904***	
Long	-0.395	-0.165	-0.173	-0.203	-0.498	0.496

Table 6.2 Spearman rank correlations of measurements made from 5-14 weeks in Experiment 1. * P < 0.05; ** P < 0.01; *** P < 0.001

Proportions of downy feathers collected from the floor ranged between 0 and 30% of the total number (overall mean 19.3%), all of which were in the short category. Numbers of downy feathers were correlated negatively with numbers of partially eaten feathers (P<0.05, Spearman rank, n=12). Partially eaten feathers accounted for between 0 and 57% (overall mean 11.6%) of feathers collected, and were present in both long and short categories.

6.2.4 Conclusions

The results of this experiment indicate an association between feather eating and feather pecking, but are not sufficient to establish a causal link. Damaging pecking was related to increased feather eating as indicated by both depletion of short feathers on pen floors and the presence of feather material in droppings. Variation in numbers of short feathers on pen floors accounted for the observed differences in feather availability, suggesting that short feathers were eaten preferentially. High levels of feather eating as juveniles were associated with outbreaks of feather pecking and cannibalism in the same groups (Pens 1,6,7 and 10 (See Section 3.3.5)) at sexual maturity.

6.3 Experiment 2- Age-related changes in feather eating and feather availability

6.3.1 Introduction

This experiment aimed to investigate age-related changes in feather eating by monitoring floor feather availability in juvenile to adult birds (from 6-26 weeks). The

study took place alongside Experiment 1a in Chapter 4. It was hoped to investigate the relationship between feather eating and feather pecking as in Experiment 1, but this was not possible since no damaging pecking occurred in either juveniles or adults.

6.3.2 *Methods*

This experiment was carried out concomitantly with Experiment 1b described in Chapter 4, and therefore the same 144 ISA Brown layer pullets were used. A description of housing and husbandry is given in Chapter 4 (Section 4.2.2). Behaviour observations were carried out from 8 weeks as in Experiment 1, except non-aggressive pecks were categorised as ‘gentle’ or ‘vigorous’ (see Section 1.1.2 for definitions). Pecking damage scores were carried out fortnightly as in Experiment 1.

From 6 to 26 weeks of age, the availability of loose feathers on the floor of each pen was estimated weekly, using the quadrat method described for Experiment 1. At 12, 18, 22 and 26 weeks the feathers were removed from the pen and measured before being returned, in all other weeks they were counted in the pen without being measured. As in Experiment 1, as feathers were being measured it was noted if they were downy or partially eaten.

At 10, 14, 18 and 22 weeks, the percentage of droppings containing feathers was determined using the method described for Experiment 1. Faecal droppings could not be examined at 26 weeks because poor litter quality made it impossible to collect individual droppings.

Proportions of droppings containing feather material and numbers of available loose feathers on pen floors at the same age were correlated. Students t tests (paired and unpaired) were carried out within and between experiments to make comparisons of floor feather counts, percentages of droppings containing feathers (after transformation to the logistic scale), and observations of behaviour.

6.3.3 *Results*

Numbers of non-aggressive pecks observed between 8-14 weeks ranged between 41 and 148 across pens (overall mean 65.5), of which an average of 24% were vigorous, while aggressive pecks ranged between 0 and 6 (overall mean 1.9). Observed feather

eating events ranged from 1 to 12 (overall mean 3.8). No pecking damage was recorded.

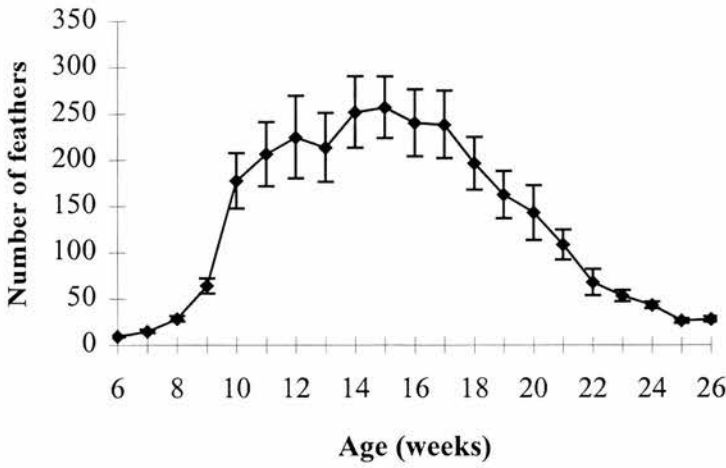
Temporal changes in the availability of loose feathers on pen floors varied between pens, but followed a pattern indicated by the mean curve (Figure 6.2a). Numbers of feathers on the floor were low at six weeks of age, ranging from 3 to 15 (overall mean 9.5). Numbers then increased slightly from nine weeks, after which large increases were observed with a maximal level of 581 feathers counted in Pen 1 at 12 weeks. Mean numbers of feathers decreased most rapidly between 18 and 22 weeks of age, and by 26 weeks had returned to low levels, ranging from 11 to 48 feathers (overall mean 27.8). Figure 6.2b shows mean changes in floor feather counts across all pens and illustrates that the biggest increases in floor feather counts occurred between the ages of nine and 14 weeks, with a steady decrease in numbers of feathers on the floor between 16 and 26 weeks. Not all pens contributed to means after 18 weeks, a water leak necessitated the changing of litter in three pens, and one pen was removed from the experiment as a result of injurious pecking.

Proportions of downy feathers on the floor tended to decrease with age (Table 6.3), and were significantly lower at 22 weeks (9.8% of collected feathers) than at 18 weeks (26%, $P < 0.01$, paired t-test, $n = 9$). At 26 weeks only 7.7% of collected feathers were downy. By contrast, proportions of partially eaten feathers increased with age (Table 6.3), from 1.2% at 12 weeks, to significantly higher levels at 18 (8.7%, $P < 0.01$), 22 (43.5%, $P < 0.001$) and 26 weeks (71.7%, $P < 0.01$).

	Mean (\pm SE) % feathers collected			
	12 weeks	18 weeks	22 weeks	26 weeks
Downy	23.9 \pm 2.4	26.2 \pm 3.9	9.8 \pm 2.8	7.7 \pm 1.5
Partially eaten	1.2 \pm 3.5	8.7 \pm 14.0	43.5 \pm 25.9	71.7 \pm 22.1

Table 6.3 Mean percentages of downy and partially eaten feathers collected at ages 12, 18, 22 and 26 weeks in Experiment 2.

a.



b.

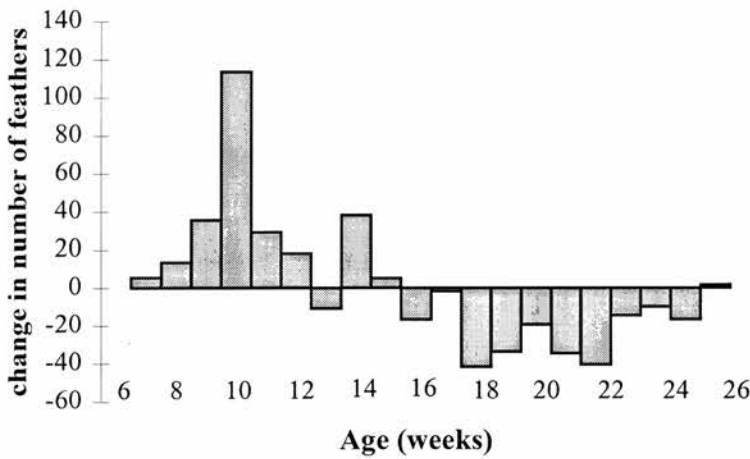


Figure 6.2 Floor feather counts from 6 to 26 weeks of age in Experiment 2; a. Mean (\pm SE) floor feather counts across all pens; b. Mean changes in floor feather counts across all pens.

At 12 weeks of age a bimodal distribution of feather length frequencies similar to that seen in Experiment 1 (Figure 6.1) was apparent. The distribution at 18 weeks (Figure 6.3a) showed a decrease in the in the availability of short feathers, but there were significantly more long feathers than at 12 weeks of age ($P < 0.01$, t-test, $n = 12$). By 22

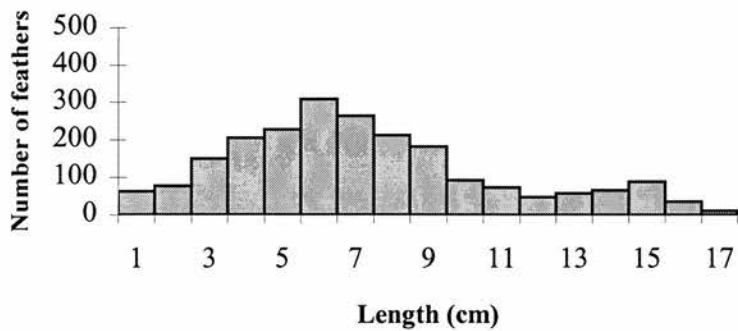
weeks (Figure 6.3b), the availability of feathers was further reduced, with significantly fewer short feathers than at 18 weeks ($P < 0.01$, t-test, $n = 12$). At 26 weeks (Figure 6.3c) levels of both short and long feathers were significantly lower than at 22 weeks ($P < 0.05$, t-test, $n = 9$ and $P < 0.01$, t-test, $n = 9$ respectively).

Proportions of droppings containing feathers tended to increase with age (Table 6.4), ranging from 2 to 21% at 10 weeks, 4 to 30% at 14 weeks, 10 to 29% at 18 weeks and 11 to 27% at 22 weeks. Sequential comparisons between the four ages showed that levels of feather material in droppings increased significantly between 10 and 14 weeks ($P < 0.001$, paired t-test, $n = 12$), but not between 14 and 18 weeks or 18 and 22 weeks. Correlations (Spearman rank) between proportions of droppings containing feather material and available loose feathers on pen floors at the same age were negative and significant at all ages, and strongest at 18 weeks.

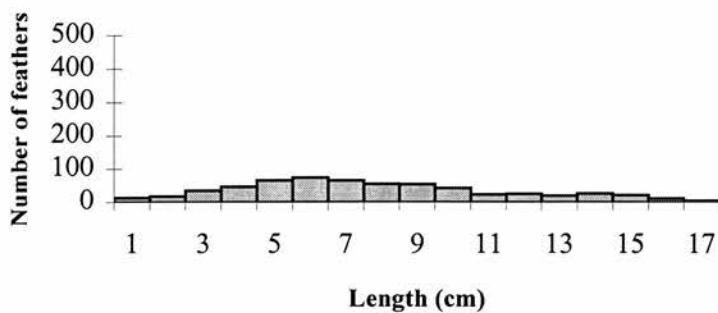
	% Droppings containing feather material			
	10 weeks	14 weeks	18 weeks	22 weeks
Pen 1	3	8	*	*
Pen 2	3	9	10	13
Pen 3	4	4	13	11
Pen 4	5	13	17	24
Pen 5	11	27	23	14
Pen 6	21	30	29	25
Pen 7	14	17	17	23
Pen 8	6	7	15	18
Pen 9	5	13	12	23
Pen 10	13	23	19	*
Pen 11	2	8	13	*
Pen 12	10	25	23	27
Mean \pm SE	8.1 \pm 1.7	15.3 \pm 2.6	17.4 \pm 1.7	19.7 2.0
Correlation (r)	-0.600	-0.821	-0.925	-0.646
P value	< 0.05	< 0.01	< 0.001	< 0.05

Table 6.4 Incidence of feather material in droppings at ages 10, 14, 18 and 22 weeks, and their correlations (Spearman rank) with counts of floor feather availability at the same age in Experiment 2. (* indicates a missing value)

a.



b.



c.

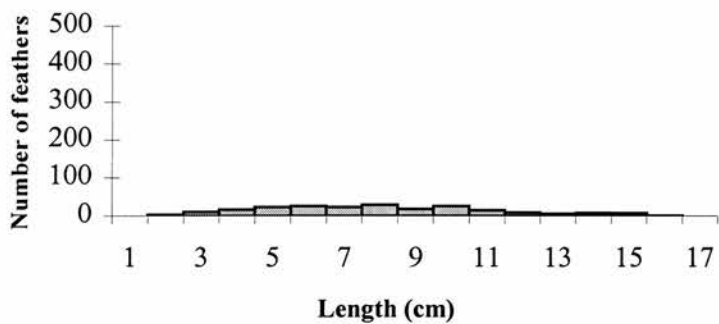


Figure 6.3 Overall frequency distribution of feather lengths from loose feathers collected from all pens at ages 18, 22 and 26 weeks in Experiment 2 at a. 18 weeks, b. 22 weeks and c. 26 weeks of age.

6.3.4 *Comparisons between Experiments 1 and 2*

There were significantly fewer total available feathers ($P < 0.05$, t-test, $n = 12$) and a higher proportion of partially eaten feathers ($P < 0.05$, t-test, $n = 12$) in Experiment 1 compared to Experiment 2 at 12 weeks of age. At 14 weeks, the difference in mean proportion of droppings containing feathers (greater in Experiment 1 than in Experiment 2) approached significance ($P = 0.084$, t-test, $n = 12$).

6.3.5 *Conclusions*

Age related changes in floor feather counts and feather material in droppings were observed. A moult at 9-11 weeks of age greatly increased the number of feathers available on pen floors, which were then depleted by feather eating over subsequent weeks. Frequency distributions of feather lengths also showed age-related changes, indicating that short feathers were eaten preferentially. Long feathers were partially eaten when short feathers became scarce. Levels of feather eating were lower in Experiment 2 than in Experiment 1, and no pecking damage was recorded.

6.4 **Experiment 3:** Feather eating : a precursor of feather pecking?

6.4.1 *Introduction*

Since no damaging pecking occurred in Experiment 2, it was not possible to relate temporal changes in feather eating and feather pecking. Experiment 1 demonstrated a relationship between levels of feather eating and pecking damage in juvenile birds, and suggested that high levels of feather eating in the growing period were associated with pecking damage after the onset of lay. However, only 4 out of 12 pens exhibited pecking damage and a relationship between feather eating and pecking damage was shown only in juvenile birds. To further investigate the association between feather eating and feather pecking, Experiment 3 aimed to relate floor feather counts and pecking damage scores at various ages. This study was carried out as part of the dietary protein source experiment described in Chapter 5, and a second aim was to determine whether dietary treatment (see Section 5.2) had any effect on levels of feather eating.

6.4.2 *Methods*

This experiment was carried out concomitantly with the study described in Chapter 5, and therefore the same 144 ISA brown layer pullets were used. A description of dietary treatments, housing and husbandry is given in Chapter 5 (Section 5.2). From 2 to 26 weeks of age, the availability of loose feathers on the floor of each pen was estimated fortnightly, using the quadrat method described for Experiment 1. Pecking damage scores collected fortnightly between 2-26 weeks of age as part of the dietary protein source experiment were utilised.

No significant differences were observed in floor feather counts or pecking damage scores between treatments ('animal' or 'plant' protein diets) so the data were pooled for further analysis. Floor feather counts and pecking damage scores at each age were correlated (Spearman rank) in a matrix.

6.4.3 *Results*

Dietary protein source ('animal' or 'plant') had no effect on levels of feather eating, as measured by floor feather counts. Pecking damage scores were recorded in all pens, the most severe feather pecking took place in Pens 2, 4, 5, 7, 10 and 12. Numbers of feathers on the floor increased most rapidly between 8-11 weeks (to a maximum of 129 feathers in Pen 8 at 12 weeks) (Figure 6.4), but never reached the high levels seen in Experiment 2.

Correlations between floor feather counts and pecking damage scores yielded 24 significant results, there were no significant correlations before 8 weeks (Table 6.5). All the correlations were negative, indicating that where there were fewer feathers on the floor, there was more pecking damage. The majority of significant correlations were found between floor feather counts in the growing period (8-18 weeks) and pecking damage after the onset of lay (18-24 weeks). Three same age correlations were significant, those at 10, 18 and 26 weeks. The strongest correlation was between feathers on the floor at 14 weeks and pecking damage scores at 22 weeks ($r_s = -0.848$, $P < 0.005$).

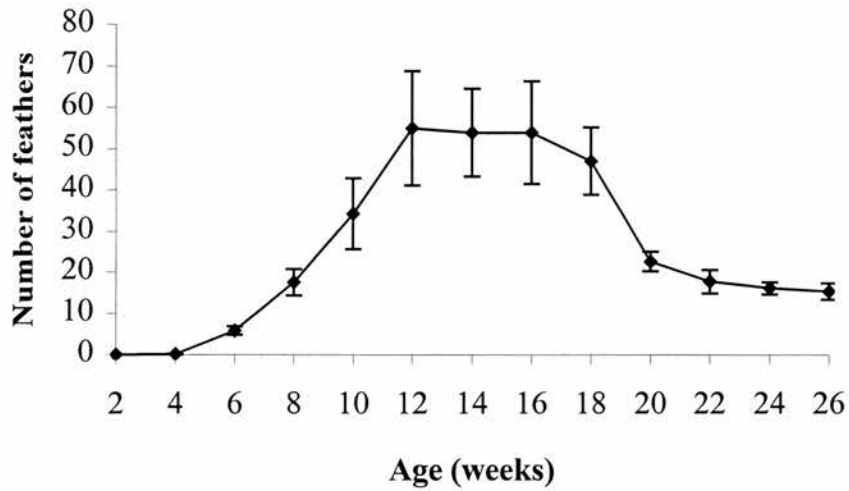


Figure 6.4 Floor feather counts (\pm SE) from 2 to 26 weeks of age across all pens in Experiment 3.

		Floor feather counts										
		Age (wks)	8	10	12	14	16	18	20	22	24	26
Pecking damage scores	8			**								
	10		*									
	12											
	14											
	16		*									
	18						*	*	**			
	20	**	**	***	**	*	*					
	22	**	**	**	***							
	24	**	*	*	*	*	*					
	26										**	**

Table 6.5 Significant correlations (Spearman rank) between floor feather counts and pecking damage scores at various ages in Experiment 3. All correlations are negative.
 * $r_s > 0.475$, $P < 0.05$ ** $r_s > 0.703$, $P < 0.01$ *** $r_s > 0.780$, $P < 0.005$

6.4.4 *Conclusions*

Floor feather counts tended to be low (as a result of feather eating) in this experiment, and moderate to severe pecking damage took place in 6 pens. Floor feather counts in the growing period were strongly correlated, negatively, with pecking damage scores at and after sexual maturity. These results provide evidence that floor feather availability in the growing period (reflecting the extent of feather eating taking place) reliably predicts later pecking damage.

6.5 **Experiment 4:** Feather eating in individually caged adult ‘pecker’ and ‘non-pecker’ hens

6.5.1 *Introduction*

The results of Experiments 1 and 3 strongly suggest an association between feather eating and damaging pecking. The measurements of feather eating used so far, floor feather counts and feather material in droppings, were carried out on a per pen basis. While direct observations of feather eating had taken place, these were relatively rare, making it difficult to examine the feather eating behaviour of individual birds. Findings in this thesis (Section 3.3.4) and elsewhere (Keeling, 1994; Savory, 1995; Savory and Griffiths, 1997) have shown that where pecking occurs, a minority of ‘pecker’ individuals are responsible. The question being addressed in this experiment was whether these ‘pecker’ individuals were also responsible for the majority of feather eating.

The experiment was split into two parts, both of which were carried out using the same set of adult, individually caged ‘pecker’ or ‘non-pecker’ hens (identified from a previous experiment). The first aim was to examine individual variation in propensity to feather eat in relation to ‘pecker’ or ‘non-pecker’ status, by providing individually caged birds a supply of suitably sized feathers. The second aim was to investigate the possibility that impregnation with preen (uropygial) oil might contribute to the attractiveness of feathers to eat. This was suggested by Savory and Mann (1997; 1999), prompted by the observation that feathers around the preen gland (where the supply of uropygial oil is likely to be liberal) are often the first to be removed when pecking damage takes place. There is growing evidence that domestic fowl can smell, and

indications that this sense can influence a diverse range of behaviours (Jones and Roper, 1997). It is possible that uropygial oil, which has a detectable odour, at least in some species (Jacob and Ziswiler, 1982), could influence feather eating behaviour. To investigate this, hens were offered a simple choice test to examine whether presence or absence of uropygial oil affected the likelihood of feather ingestion. An additional aim was to determine whether any detected preference differed between ‘peckers’ and ‘non-peckers’.

6.5.2 *Methods*

Experiment 4a Are feather peckers also feather eaters?

Sixteen 35 week-old laying hens were used in this study, which had previously been used in the experiment described in Chapter 4. ‘Peckers’ were defined as birds from pens where pecking damage had taken place, and which were known from direct observation to engage in damaging bird to bird pecking. ‘Non-peckers’ were individuals taken at random from groups where no pecking damage had occurred (thus ensuring they were neither peckers nor peckees). Every attempt was made to control for previous treatment (dietary protein source, see section 5.2 for treatment details). Of the eight non-peckers, four had previously been on the plant protein treatment (from Pen 1) and four had been on the animal protein treatment (from Pen 6). The peckers were obtained from three groups, Pen 4 (four birds), Pens 10 and 11 (one bird each) and Pen 12 (two birds). Of the eight peckers, five had previously been on the plant protein treatment and three had been on the animal protein treatment. The birds were housed individually in sloping wire floor cages (width 30 cm, height 43-50 cm, depth 46 cm) in the middle row of a three tier battery, four weeks before the experiment began. Birds were placed at random order in alternate cages, so they had visual, but not physical access to their neighbours. *Ad libitum* access to food (standard layer pellets) and water was provided via a food trough at the front of the cage and nipple drinkers to the rear of the cage.

Every bird was tested individually for propensity to feather eat in four separate trials, carried out on Tuesdays and Thursdays in two subsequent weeks. Feathers were plucked from the back, tail and wings of freshly killed hens of the same age and strain, from which semiplumes between 4 and 6 cm in length were selected. Feathers from four

birds were used, each bird providing the feathers for one trial. Each test lasted 10 minutes, and birds were tested in random order. The test situation consisted of an observer sitting adjacent to and 1m to the left of the home cage. At test, one feather was placed on top of the food in front of the test bird's home cage and a stopwatch was started. If the presented feather was eaten, the latency to eat the feather was recorded, and another feather was immediately placed in front of the bird. This process was repeated, recording all feathers ingested in the 10-minute test period. If the feather was not eaten it remained in front of the bird until the end of the test. If it was picked up and dropped by the bird so that it was out of reach (through the wire floor of the home cage, for example) it was immediately replaced. As well as feather ingestion, numbers of pecks (pecking at the feather without picking it up), 'pick-ups' (picking up the feather without immediate subsequent ingestion), and manipulations (manipulation of the feather in the beak, with or without subsequent ingestion) towards the presented feather were recorded. During each trial, general comments on the test bird's behaviour, such as whether it ate food or drank, were recorded.

Total counts of feather ingestion, pecking, pick-up and manipulation were calculated across the four trials. The counts obtained were not normally distributed despite log transformation and could not meet the assumptions required by tests to compare two means. Therefore, a random permutation test (Siegel, 1956), which makes no assumptions about the nature of the data, was carried out to calculate the statistical significance of the difference between pecker (P) and non-pecker (NP) birds. This test uses a random sample of 1000 different allocations of P and NP to the data, eight of each as in the original experiment. The significance probability is the proportion of these allocations that give a mean difference between P and NP at least as great in magnitude (regardless of sign) as that which was actually observed. Mann-Whitney U tests were carried out between trials to investigate variation in feathers eaten from the four donor birds.

Experiment 4b Does the presence/absence of preen oil affect feather eating?

This experiment took place two weeks after Experiment 4a, with the same birds maintained in the same cages and positions as previously described. Feathers were

plucked from the back, tail and wings of a single freshly killed hen of the same age and strain, which had been housed in a pen with access to litter. As before, semiplumes between 4 and 6 cm in length were selected. Roughly half of the feathers were washed (to remove uropygial oil) as follows. The feathers were plunged into 70% ethanol (at room temperature) for 5 minutes, stirred occasionally, drained, rinsed with hot water, thoroughly rinsed with cold water, placed on absorbent paper and left to dry at room temperature. There was no discernible visible difference between washed and unwashed feathers once dry, but the author could detect a difference in odour between washed and unwashed feathers. Washed and unwashed feathers were placed in sealed plastic bags until required.

Birds were tested individually and in random order in two separate trials. At test, each bird was presented with two separate, identical white plastic semicircular dishes (diameter 9cm) in front of the home cage, for a 10 minute period. One dish contained 20 washed (W) feathers, and the other 20 unwashed (U) feathers. During the test period, numbers of feathers ingested from each dish were recorded. Observations of 'pick-ups' or pecks at feathers from either dish were also recorded in the same way as Experiment 4a. The left/right positioning of dishes of washed and unwashed feathers was randomised to control for side preferences, and the positioning of dishes in Trial 2 was the opposite of that in Trial 1. The position of the observer (to the left or right of the home cage) was randomised for both trials. Total counts of feather ingestion, 'pick-ups' and pecks were calculated across the two trials. Proportions of unwashed feathers (P^U) eaten, pecked or picked up were calculated as follows: $P^U = U/(U+W)$. Therefore, a P^U value of 0.5 indicates no preference (neutral), while values >0.5 indicate a preference for unwashed feathers, and values <0.5 indicate a preference for washed feathers. P^U values for feather ingestion, pick-ups and pecks were compared to neutrality (0.5) using one sample t-tests. In the feather ingestion analysis, birds eating very few or no feathers (less than 5% feathers offered, i.e. fewer than four feathers) were excluded, to avoid introducing bias into the P^U mean where birds showed little or no interest in the feathers. All 16 birds were included in analysis of pecking and picking-up. P^U values for pecker and non-peckers were compared with two-sample t-tests, as were total feathers eaten, pecked and picked up.

6.5.3 Results

Experiment 4a

Several birds readily ate the presented feathers, and 14 out of 16 birds did so on one or more occasion (Plate 6.2). Wide variation between birds was observed in the numbers of feathers eaten, pecked, picked-up and manipulated (Table 6.6). Latencies to eat the first feather presented ranged from 4 s to 192 s (where a feather was eaten). Peckers ate, picked-up and manipulated feathers significantly more than non-peckers (Random Permutation test, $P < 0.05$, $P < 0.01$, $P < 0.01$, respectively, Table 6.6). There was no significant difference between peckers and non-peckers in pecking at presented feathers. The two birds eating no feathers were both non-peckers, while the three birds eating the highest number of feathers were peckers.

Cage	P/NP	Eaten	Latency (s)	Peck	Pick-up	Manipulate
2	P	155	11	18	24	214
4	P	18	329	7	17	45
6	P	119	55	45	59	302
7	P	49	158	20	14	116
9	P	41	27	43	53	201
14	P	118	10	38	45	224
15	P	42	42	151	96	307
16	P	93	16	82	50	267
1	NP	1	454	17	13	9
3	NP	0	*	11	3	1
5	NP	0	*	38	18	29
8	NP	8	176	12	25	44
10	NP	64	12	26	16	177
11	NP	115	15	28	23	241
12	NP	6	323	57	32	120
13	NP	29	50	22	26	101
Mean	P	79.4	80.9	50.5	44.8	209.5
	NP	27.9	171.5	26.4	19.5	90.3
P value (Permutation test) $P < 0.05$				$P = 0.19$	$P < 0.01$	$P < 0.01$

Table 6.6 Total numbers of feathers eaten (with mean latency to eat first feather), pecked, picked-up and manipulated by peckers and non-peckers in Experiment 4a.

* indicates that no feathers were eaten



Plate 6.2 An individually caged hen ingesting a presented feather

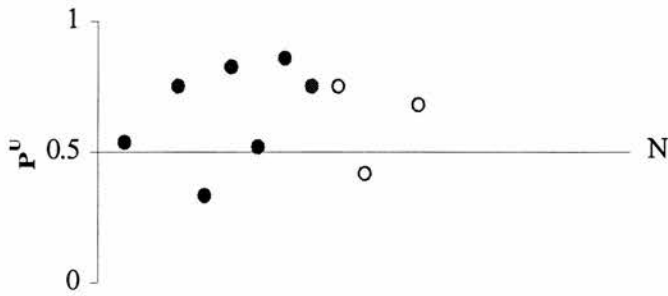
It was noticed that birds turned and manipulated the presented feather to line up with the beak lengthways, quill end pointing into the mouth (Plate 6.2), before swallowing. Larger feathers (length about 6cm) were manipulated for longer (moved in the beak with a characteristic side-to-side motion, hit against the food, shaken) before being ingested. Smaller feathers (length 4-5cm) tended to receive less manipulation, sufficient to ensure the preferred orientation before the feather was ingested. Some birds turned to face the back of the cage before ingesting feathers. Drinking from the nipple drinker after feather eating was observed on six occasions. No significant differences were observed in feathers eaten between trials, suggesting that no donor bird or day effect was present.

Experiment 4b

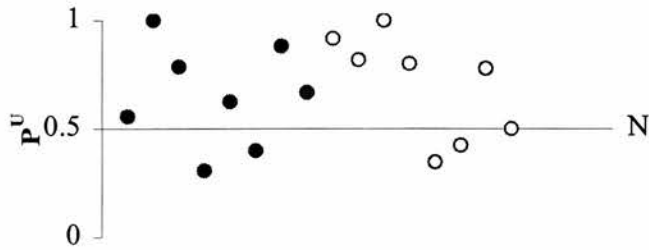
Feathers presented in dishes were eaten, with 14 out of 16 birds ingesting at least one feather, and all birds pecking or picking up feathers. Across peckers and non-peckers, unwashed feathers elicited significantly more interest in terms of eating ($P < 0.05$, $n=10$, Figure 6.5a), pecking ($P < 0.01$, $n=16$, Figure 6.5b) and pick-ups ($P < 0.01$, $n=16$, Figure 6.5c).

There was no significant difference between peckers and non-peckers in the level of preference for unwashed feathers (P^U) for eating, pecking or picking up. While numbers of feathers eaten, pecked or picked up overall (across washed and unwashed) by peckers and non-peckers were not significantly different, it should be noted that seven out of eight peckers ate at least 5% of feathers presented, compared to only three out of eight non-peckers.

a.



b.



c.

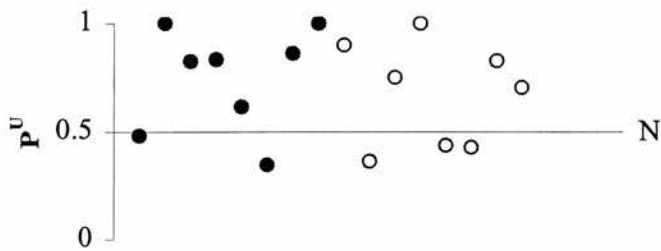


Figure 6.5 P^u (>0.5 indicates a preference for unwashed feathers) expressed by peckers (closed circles) and non-peckers (open circles) for a. eating (mean $P^u=0.64$, $P<0.05$); b. pecking (mean $P^u=0.68$, $P<0.01$); and c. picking-up (mean $P^u=0.71$, $P<0.01$) in Experiment 4b. The points are arbitrarily spaced along the x-axis for clarity. N= neutrality (i.e. no preference)

6.5.4 *Conclusions*

Experiment 4a demonstrates that adult hens will readily eat small feathers (4-6cm), even when they are presented in a somewhat artificial situation. Pecking status had a significant effect on propensity to feather eat: peckers ate, picked up and manipulated more feathers than non-peckers. Birds manipulated feathers to a preferred orientation in the beak (in line with the beak, with the quill end pointing into the beak) before ingestion.

Unwashed feathers (containing uropygial oil) were eaten, pecked and picked-up in preference to washed feathers, by both peckers and non-peckers. There was no effect of pecking status on the level of preference for unwashed feathers, but it appeared that, as in Experiment 4a, peckers ate presented feathers more readily than non-peckers.

6.6 **Discussion**

In Experiment 1, when pecking damage occurred in 5 of the 12 pens, there was no significant correlation between damage scores and numbers of non-aggressive pecks observed. This is probably because most of the latter are gentle and non-damaging, (Savory, 1995). If gentle pecks and vigorous pecks/pulls had been recorded separately, the damage scored in some pens might have been reflected by higher counts of the vigorous type. The highly significant correlation found between pecking damage scores and numbers of aggressive pecks observed in Experiment 1 was unexpected, and may be misleading. Aggressive pecks accounted for only a small proportion of total pecks observed, and the correlation is strongly influenced by the fact that 2 pens (1 and 6) had the highest levels of both pecking damage and aggressive pecking (Table 6.1). Previous work investigating the relationship between feather pecking and social rank was inconclusive (Wood-Gush & Rowland, 1973).

In Experiment 1, numbers of observed feather eating events were low and variable, and as a result were not correlated significantly with feather material in droppings or floor feather counts. Where feather eating was observed, the floor litter was the most common source of eaten feathers, followed by feathers caught in the air as they floated to the ground, particularly after some disturbance in the pen caused them to be thrown into the air. A few birds were seen to eat their own feathers immediately after

they were dislodged by preening, and on one occasion a feather was eaten after it had been stolen from a pen-mate.

All feather sizes were found to be partially eaten, except very small (1-2cm) and completely downy feathers. There were fewer downy feathers in pens where feather eating was most common, but since downy feathers tended to fall into the 'short' category, it is difficult to ascertain whether a preference for downy feathers over other types of short feathers was present. It is possible that downy feathers, which tend to have a softer rachis, were easier to swallow and were therefore preferred.

The changes observed in feather length frequency distributions with age in Experiment 2 support the assertion that short feathers are eaten preferentially, since this was clearly the part of the distribution curve which was most affected over time (Figure 6.3). The distributions at 22 and 26 weeks also show reductions in the numbers of long feathers available, but this can be explained by the prevalence of partially eaten feathers at these ages (ranging from 33% to 100% of all feathers measured at 26 weeks). Feathers (intact or otherwise) were measured as they were collected, with no attempt made to estimate their original size. Long feathers, for example, originally measuring 14-16 cm, could be measured as 8-10cm fragments, with only the thickest (and stiffest) part of the quill (calamus) and rachis remaining (Plate 6.1).

The rate at which feathers were cast by birds in Experiment 2 could not be measured, making it impossible to determine a rate of feather eating on the basis of floor feather counts. However, it seems reasonable to assume that moulting rate would have been roughly equal across pens containing the same number of birds. The moult resulting in the largest increase of loose feathers was age related (occurring between 9 and 11 weeks), but it is possible that slight differences in the rate of development of individuals could have caused variation in feather availability. Litter turnover and bird movement could possibly have affected floor feather counts, since only feathers on the litter surface were counted.

In Experiments 1 and 2, levels of feather material detected in droppings were higher than levels reported previously for bantams (Savory & Mann, 1999). Feathers that had passed through the digestive system often had a yellow appearance making them distinguishable from uneaten feather material, and care was taken to ensure that

only feather material contained within faecal fragments was counted. It is likely that the proportion of droppings containing feathers was an underestimate, since very small feathers were not detectable in droppings. In Experiment 2, levels of feather material in droppings increased with age (Table 6.4), presumably reflecting the cumulative nature of the measurement. Thus, the droppings examined could have been produced at any time prior to collection, and would not necessarily reflect the amount of feather eating then. Nevertheless, strong negative correlations between feather material in droppings and floor feather availability were observed at all ages (Table 6.4), suggesting that feather eating continued throughout the experiment and accounted for the observed depletion of feathers on the floor.

The pecking damage seen in juveniles in Experiment 1 was not severe, and occurred mainly between 5 and 10 weeks of age, as reported previously (Hughes, 1973). Damaging pecking at this age tends to be directed at the tail and preen gland, and the back region in front of the preen gland is usually the first part of the body to be denuded of feathers (Savory & Mann, 1999). The feathers there are accessible, easily plucked, of a size preferred for eating (2-6cm), and a liberal supply of preen oil due to their proximity to the preen gland may make them especially attractive.

Comparisons between Experiments 1 and 2 can only be carried out to 14 weeks of age. In Experiment 2, no feather pecking or cannibalism took place, but high levels of aggressive pecking after the onset of lay led to some 'bullied' birds being culled (as a result of pecking injuries to the head, neck or comb or weight loss, see Section 4.2.3). Feather eating took place in both experiments, but reached higher levels in Experiment 1. In Experiment 2, there were more loose feathers available at 12 weeks of age, less feathers in droppings and less partially eaten feathers than in Experiment 1. An overall low availability of moulted feathers in pen floors was associated with a high incidence of pecking damage (present in all pens) in Experiment 3. This suggests a possible threshold effect, where limited availability of loose feathers to eat at a particular age may redirect attention towards the feathers of pen mates.

These results support the notion that feather eating may be a 'precursor' to subsequent damaging pecking. In Experiment 1, the pens with the highest levels of feather eating and pecking damage as juveniles were also those in which outbreaks of

feather pecking and cannibalism occurred around the onset of lay. In Experiment 3, reduced availability of feathers on pen floors during the growing period (as a result of feather eating) reliably predicted the occurrence of pecking damage in adulthood.

While Experiments 1, 2 and 3 concentrated on feather eating in groups of birds, Experiments 4a and 4b examined propensity to feather eat in individuals. The finding that birds identified as peckers ate more feathers than non-peckers suggests that these members of groups may be responsible for the majority of feather eating as well as feather pecking. While peckers picked-up and manipulated feathers more than non-peckers, no difference was observed in numbers of pecks at the presented feather. It may be the case that a single feather does not elicit pecking in the same way as the feathers of another bird. No attempt was made to distinguish between gentle and vigorous pecks at presented feathers, which may have differed between peckers and non-peckers. The hunger state of the birds prior to the trials in this study was not known. It is unlikely that the birds ate feathers because they were hungry, since they had *ad libitum* access to food at all times, including during the experimental trials.

In Experiment 4b, both peckers and non-peckers showed a preference for unwashed feathers to eat, peck and pick-up. This could indicate an attraction towards unwashed feathers, or an avoidance of washed feathers for some reason. Care was taken to wash the feathers in a manner that would be unlikely to introduce new olfactory stimuli, and to ensure that the visual and physical properties of the feathers were unchanged by the washing procedure. While this appeared to be the case in terms of human olfaction (the washed feathers had no discernible odour, the unwashed ones did) and vision (the feathers looked identical), feather properties undetectable to humans may have been affected. Clearly, birds would not encounter washed feathers normally, and the results may reflect neophobia, which is commonly exhibited by chickens (Murphy, 1977; Jones, 1987). Chickens have been shown to be attracted to familiar odours previously (Jones and Faure, 1982), and the results may indicate a preference for a familiar (conspecific) odour over no odour.

Extractions from feathers of domestic fowl have shown that feathers are coated with preen oil (Bolliger and Varga, 1961). Suggested functions of preen oil include water repellence, maintenance of general plumage hygiene, maintenance of feather

flexibility, and a possible source of vitamin D (Jacob and Ziswiler, 1982). In the domestic fowl, preen oil consists mainly of diester waxes (composed of unbranched fatty acids), with triglyceride and cell fragments (Haati and Fales, 1967). The preen gland is considered a scent gland by some authors (Rawles, 1960), and growing evidence suggests that duck preen oil has pheromonal properties relating to the regulation of reproductive behaviour (Jacob et al., 1979; Kolattukudy et al., 1987). Wurdinger (1982) demonstrated that goslings chose to sleep in warming boxes impregnated with a familiar smell, including conspecific preen oil. In ducks, intermediate length 3-hydroxy fatty acids are thought to be the pheromonal component of preen oil (Jacob and Ziswiler, 1982). These particular compounds have not been detected in galliform preen oil, but related unbranched fatty acids are present which could have similar properties. While studies on domestic chicks have demonstrated attachment to artificial odours such as orange or geranium oil (Jones and Gentle, 1985), olfactory properties of preen oil have not been investigated in chicks or adults. The results of Experiment 4b provide circumstantial evidence that preen oil may play a role in attractiveness of feathers to peck and eat, since hens could clearly distinguish between feathers treated in such a way as to alter their olfactory properties. An experiment offering birds feathers supplemented with additional preen oil could ascertain to what extent preen oil enhances the attractiveness of feathers.

In Experiment 4b, while peckers ate more feathers than non-peckers (seven out of eight peckers eating four or more feathers compared to three out of eight non-peckers), the difference was not significant. There were major differences between Experiments 4a and 4b in the way in which feathers were presented to the birds. In Experiment 4a, single feathers were presented, with action required by the bird (ingestion of the feather) before another became available. Several feathers (40) were presented simultaneously in Experiment 4b to offer a choice and also to concentrate any olfactory cues. It is possible that a single feather has a different stimulus value than several. The repeated presentation method used in Experiment 4a may also have had an effect, due to the fact that birds were interacting with the observer/feather provider. If such an effect did exaggerate the difference between peckers and non-peckers in Experiment 4a, this may still indicate underlying differences in peckers and non-peckers

‘personalities’, or perhaps fear state. Despite an interval of three weeks between Experiments 4a and 4b, it is also possible that previous exposure to feathers could have affected the results of Experiment 4b. The results of Experiment 4 should be treated with caution, since only small numbers of birds were involved and they were re-used. This type of study is hindered by the unpredictability of feather pecking behaviour and consequent difficulty in obtaining known peckers.

Collectively, these experiments provide convincing evidence for a relationship between feather eating and feather pecking. Feather eating appears to be common in juvenile and adult commercial layers, and the demonstrated associations between bird to bird pecking and feather ingestion in groups and individuals indicate that feather eating could be an important contributory factor in the development of damaging pecking. It is still not fully understood why feathers are so attractive and readily eaten. The results of Experiment 4b suggest that the presence of preen oil on the feather surface may be involved, but it is likely that olfactory properties are just one component of the attractiveness of feathers to eat. It is not yet clear whether feather eating promotes feather pecking, or whether feather eating is reinforced through ingestion of feathers removed from other birds. Feather eating begins in the growing period, following a juvenile moult, and it is possible that once feather eating has become established, a low availability of suitably sized moulted feathers may cause feather eating and pecking to be redirected towards other birds. This has relevance for the egg industry, since commercial layer pullets are usually reared on litter floors, with access to moulted feathers, regardless of housing in adulthood.

Feather pecking is characterised by its unpredictable occurrence and only a few behavioural correlates have been identified (Keeling and Jensen, 1995; Savory and Griffiths, 1997). Even if previous feather eating is not a primary cause of damaging pecking, the strong association between the two behaviours is an important finding. These results have shown that levels of feather eating in juveniles, before any pecking damage takes place, are directly related to subsequent pecking damage. A simple approach such as monitoring floor feather availability could be of use in breeding programs aimed at suppressing pecking problems.

Chapter 7

General discussion

This project aimed to investigate factors influencing the aetiology of feather pecking and cannibalism in commercial layer pullets. Emphasis was placed on internal factors (intrinsic developmental changes and hormonal changes at sexual maturity), but the role of external factors (dietary protein source and feather eating) was also examined.

The findings described in this thesis confirm that the development of feather pecking and cannibalism in layer pullets follows a consistent pattern. There is a temporary peak in potentially damaging pecking activity in juveniles between 5 and 10 weeks of age, followed eventually by an increase in more severe types of pecking at the onset of lay (at about 16 weeks). This pattern agrees with previous observations of the development of damaging pecking (Hughes and Duncan, 1972; Hughes, 1973; Allen and Perry, 1975; Kjaer and Sorensen, 1997; Huber-Eicher and Wechsler, 1997; Bilcik and Keeling, 1999). Importantly, there was a strong association (on a per group basis) between the existence of damaging pecking in juveniles and the occurrence of severe pecking damage after the onset of lay. This was because the same individuals tended to cause pecking damage in the growing period and also after the onset of lay. The physiological basis of the peak in damaging pecking in juveniles is not known, and merits further investigation. More detailed physiological information from this period in development is required, since in the studies described in this thesis, emphasis was placed on developmental changes towards sexual maturation, from 10 weeks of age onwards. Whatever its cause, the fact that damaging pecking in juveniles can reliably predict later pecking is a useful piece of knowledge that could be used in future studies on feather pecking to aid experimental design and targeting of treatments. Changes in the extent of pecking damage were related to age related changes in types of bird to bird pecks, with damaging pecks and pulls seen to a greater extent after the onset of lay (Chapter 3). In agreement with previous studies (Hughes and Duncan, 1972), cannibalism was seen only after sexual maturity. Age-related changes in targeting of pecks towards

different parts of the body were also apparent, with the tail being the most common target in juveniles. Pecks tended to be directed towards the base of the back and preen gland at the start of lay, while vent pecking was more common later. Unexpectedly, large increases in aggressive pecking at the onset of lay were also observed, which, in some experiments, were seen more reliably than feather pecking. In some cases, aggressive attacks were severe, resulting in deaths or culls. Such severe aggression has not been reported previously, but Bilcik and Keeling (1999) reported an increase in pecking damage to the head (which was attributed to aggressive pecking) at the onset of lay. The extent to which this kind of aggressive behaviour is a problem in commercial flocks is not clear, since the term 'aggression' is commonly used in the egg industry to describe all forms of injurious pecking. Similar bullying of particular birds, which seem to attract aggression by their constant submissive behaviour, has been observed in flocks of 100 birds in experimental perchery modules (C.J. Savory, personal communication, 1999).

The age-related behavioural and hormonal changes seen in Chapter 3 served as a baseline and provided circumstantial evidence for hormonal involvement in increased pecking damage at the onset of lay. Unfortunately, attempts to repeat and extend the work carried out by Hughes (1973) were unsuccessful, because of difficulties encountered in reliably manipulating hormonal state with chronic administration of exogenous oestradiol and progesterone. Hughes used pure crystalline hormone pellets, which could not be used in this project because they are no longer manufactured due to safety concerns. Since it is unlikely that the commercial pullets used in the present project were physiologically different to those used by Hughes in 1973, the problems encountered were probably due to the type of implants used, which had poor/variable release rates and were rapidly encapsulated. Although some hormonal manipulation was achieved (Chapter 4), it was acute and/or showed large variability between individuals. An important point to consider is that in the original work, Hughes may also not have achieved the prolonged release of hormone his crystalline pellets advertised, and since hormonal state was not monitored in his experiment, he had no indication of their reliability. Hughes's results are consistent with a large dose of hormone, for example he reported long term effects on egg production in oestradiol/progesterone treated birds. However,

results in this thesis and elsewhere have demonstrated that acute large doses hormone can have long-lasting effects, and do not necessarily relate to continued hormone release. The lack of physiological data relating to Hughes (1973) experiment means that no basis for comparison is available, and this, coupled with less effective implants, probably explains the failure to replicate the original study. Nevertheless, previously unavailable information about the performance of various methods of administration of hormones in birds was obtained in this project, which could be of use in future work.

Results obtained in this thesis (Chapter 3) and elsewhere (Hughes, 1973) suggest that progesterone may be of greater importance in the control of pecking behaviour than oestradiol. Almost nothing is known about the effects of progesterone on avian behaviour (other than nesting and incubation behaviour). This makes it difficult to construct a hypothesis for the potential involvement of progesterone in the development of pecking damage. A few studies have demonstrated effects of oestradiol on the behaviour of domestic fowl (increasing activity, for example). Given the known cooperativity between oestradiol and progesterone receptors (Kawashima et al., 1979; Kamiyoshi et al., 1992; Kawashima et al., 1992), it is likely that if increased damaging pecking at the onset of lay has a hormonal basis, both hormones act together to alter behaviour.

A second approach to the manipulation of hormonal status of the birds was employed. This consisted of the application of an antagonist to block endogenous oestrogen effects. Acute administration of the anti-oestrogen tamoxifen resulted in a reduction in vigorous pecking and pulling (but not gentle pecking and pulling) at a novel object (a bunch of string) in adult hens (Chapter 4). The suppressive effects of tamoxifen on vigorous pecking and pulling indicate an oestradiol receptor mediated control of propensity to peck, but the exact mechanism by which tamoxifen exerts its effects is not clear, due to the paucity of information about effects of gonadal steroids on behaviour in the domestic fowl. These results add weight to the hypothesis that gonadal hormones are involved in the control of pecking behaviour, but were based on a small number of birds pecking at an inanimate object. An obvious next step would be to investigate the effects of tamoxifen on bird to bird pecking in a group situation. The results of Chapter 4 demonstrated that successful chronic tamoxifen

administration can be achieved with Silastic implants, and this methodology has potential as a tool to investigate the putative role of gonadal hormones in the development of damaging pecking. It could be argued that the observed effects of tamoxifen on pecking behaviour are attributable to concurrent endogenous production of testosterone. Androgen status has been implicated in the control of some aspects of behaviour in fowl, with administration of testosterone increasing avoidance of alarming or novel stimuli (Jones and Andrew, 1992) and reducing distractibility (Andrew and Jones, 1992). Thus, elevated plasma testosterone as a result of tamoxifen treatment could have increased avoidance of the novel pecking device, reducing pecking, or could have increased pecking by increasing attention towards the device. Hughes (1973) reported a suppressive effect of chronic testosterone administration on feather pecking, and the effects of testosterone on pecking at a device in the absence of tamoxifen could be tested relatively simply by administration of exogenous testosterone, perhaps by acute subcutaneous injection.

One of the aims of this project was to investigate individual differences in hormonal state and relate these to pecking behaviour. This approach was difficult in practice, because of the physical constraints on sampling large numbers of birds and the unpredictability of damaging pecking. When individual comparisons were made in adult hens, there was no evidence of a correlation between numbers of bird to bird pecks delivered and basal plasma concentrations of oestradiol, progesterone or testosterone (Chapter 5). No differences in basal hormone concentrations between birds identified as peckers and non-peckers were apparent (Chapter 4). It is possible that physiological differences would be apparent at other ages, or that behavioural changes are responses to rates of change in plasma hormone concentrations, rather than absolute levels, as suggested by Hughes (1973). Repeated blood sampling of individuals with known pecking status (possibly identified from observations of pecking behaviour while juvenile) up to and during the onset of lay would be required to determine unequivocally whether basal differences in hormonal state exist.

Chapter 5 investigated the role of dietary protein source in the development of damaging pecking. The results of an experiment comparing diets based on animal (fishmeal) or plant (soyabean meal) protein provided some evidence to support

producers' claims that increased pecking problems are associated with diets composed entirely of plant protein. Higher numbers of vigorous, potentially damaging pecks were seen in groups receiving the plant protein diet, but it remains unclear whether the observed effects on pecking behaviour were due to an absence of a beneficial nutrient in animal protein or the presence of a detrimental substance in plant protein. In the dietary protein experiment described here, pecking damage occurred in both animal and plant protein groups, indicating that provision of fishmeal does not eradicate pecking problems and that other causal factors are involved.

The presumed presence of phytoestrogens in the plant protein diet did not appear to affect hormonal state, although effects on plasma oestradiol of diets with soya contents higher than those used in the experiment described in Chapter 5 have been reported in laying hens (Maurice et al., 1979; Akiba et al., 1982). It remains possible that altered hormonal state as a result of dietary phytoestrogens, coupled with evidence for oestrogen mediated control of pecking behaviour, could represent a mechanism by which plant protein based diets could influence pecking behaviour. Several types of phytoestrogens exist (e.g. isoflavones, lignans) which vary in potency, and levels of phytoestrogen differ between plant species and genetic strains (Knight and Eden, 1995). In some species, phytoestrogen content is also affected by factors such as climate and geographical location (Leopold, 1976). As a result the oestrogenic potency of diets may vary with formulation or even batch. If phytoestrogens are causally related to increasing pecking damage, this variation could account for some of the unpredictability seen in pecking.

The somewhat ambiguous effect of dietary protein on damaging pecking observed may be due to the relatively small numbers of birds used, and a similar experiment, conducted on a commercial scale, could provide a clearer indication of the extent of the influence of dietary protein on the development of damaging pecking. To determine the extent to which plant protein based diets are capable of influencing hormonal state, experiments comparing the development and behaviour of pullets reared on diets containing different levels of soyabean meal should be carried out. Quantification of the oestrogenic constituents of such diets, along with

comparisons of different soybean sources, would give an indication of the degree of variability of phytoestrogen content.

Interest in feather eating behaviour and its possible role in the development of feather pecking began when it was noticed that numbers of moulted feathers on the floor were lower in pens where pecking damage was taking place. Examination of floor feather counts (cast during a juvenile moult around 10 weeks of age) and feather material in droppings demonstrated that levels of feather eating in juveniles was related not just to damaging pecking at that time, but reliably predicted the onset of more severe pecking damage at the onset of lay (Chapter 6). In pens where high levels of feather eating were taking place, complete depletion of short feathers coincided with the onset of lay and increased pecking damage. This suggests that feather eating may be reinforcing, and a low availability of moulted feathers on the floor may encourage birds to obtain feathers from pen-mates (see Figure 7.1 where floor feather availability is related to feather eating). Short feathers were preferred for feather eating, and such a preference could account for the targeting of feather pecks and pulls at areas of the body where suitably sized short feathers are easily obtained (such as the base of the back).

Experiments with individually caged hens showed that peckers had a higher propensity to eat feathers than non-peckers (Chapter 6), so it is likely that these birds were responsible for most of the feather eating (as well as pecking) in the groups where pecking damage occurred. Another experiment with individually caged hens provided some evidence that preen oil contributes to the attractiveness of feathers to eat, along with size and probably texture (downy feathers appear to be preferred). The putative attractiveness of preen oil could account for pecking targeted at the preen gland observed at the onset of lay (Chapter 3) as also reported previously (Savory and Mann, 1997). It could also be the case that intrinsic differences in preen oil composition may make certain individuals more attractive targets for pecking (or as a source of feathers to eat), although this possibility was not investigated in the current project. The attractiveness of preen oil and its role in the development of feather pecking could be further investigated by carrying out preference tests, offering birds a choice between normal feathers and feathers supplemented with

preen oil, and similar choice tests could be used to examine the differences between individuals in attractiveness of preen oil.

The most important question raised by the studies of feather eating (Chapter 6) is whether feather eating reinforces feather pecking or vice versa (see Figure 7.1). Do birds begin by feather eating and become feather peckers, moving to pen-mates as a source of feathers as moulted feathers become unavailable? Or is feather eating simply an activity more prevalent in birds 'genetically' preprogrammed to be feather peckers? Importantly, damaging pecking is seen in juveniles before the juvenile moult (i.e. before a large number of loose feathers are available on the floor), suggesting that feather pecking originates without feather eating. The appearance of feathers during the juvenile moult may direct the attention of peckers away from pen mates, and it is possible that the continuing availability of feathers during the growing period suppresses damaging pecking (while reinforcing feather eating). A reduction in pecking damage scores in the weeks following the juvenile moult was seen in two experiments, in Chapter 3 and Chapter 5. In the same experiments, increases in pecking damage coincided with complete depletion of feathers on the floor. At present, many pullets are reared on litter, with access to moulted feathers. If feather eating is an activity which can divert feather peckers from damaging pecking, it is possible that constant availability of suitably sized loose feathers could direct attention away from other birds and suppress damaging pecking. An obvious experiment to test this notion would be to rear pullets with and without a supply of loose feathers while monitoring the development of pecking damage.

The results of this project have led to the development of a tentative model describing the involvement of gonadal hormones in the development of damaging pecking, illustrated in Figure 7.1. It is proposed that changes in peripheral gonadal hormone concentrations during sexual maturation could increase pecking damage by three routes; by acting directly on propensity to peck, by increasing the performance of behaviour (preening and dustbathing) that affects propensity to be pecked, or by influencing the attractiveness of feathers by altering preen oil composition. These pathways are described in more detail below and are marked with numbered arrows in Figure 7.1.

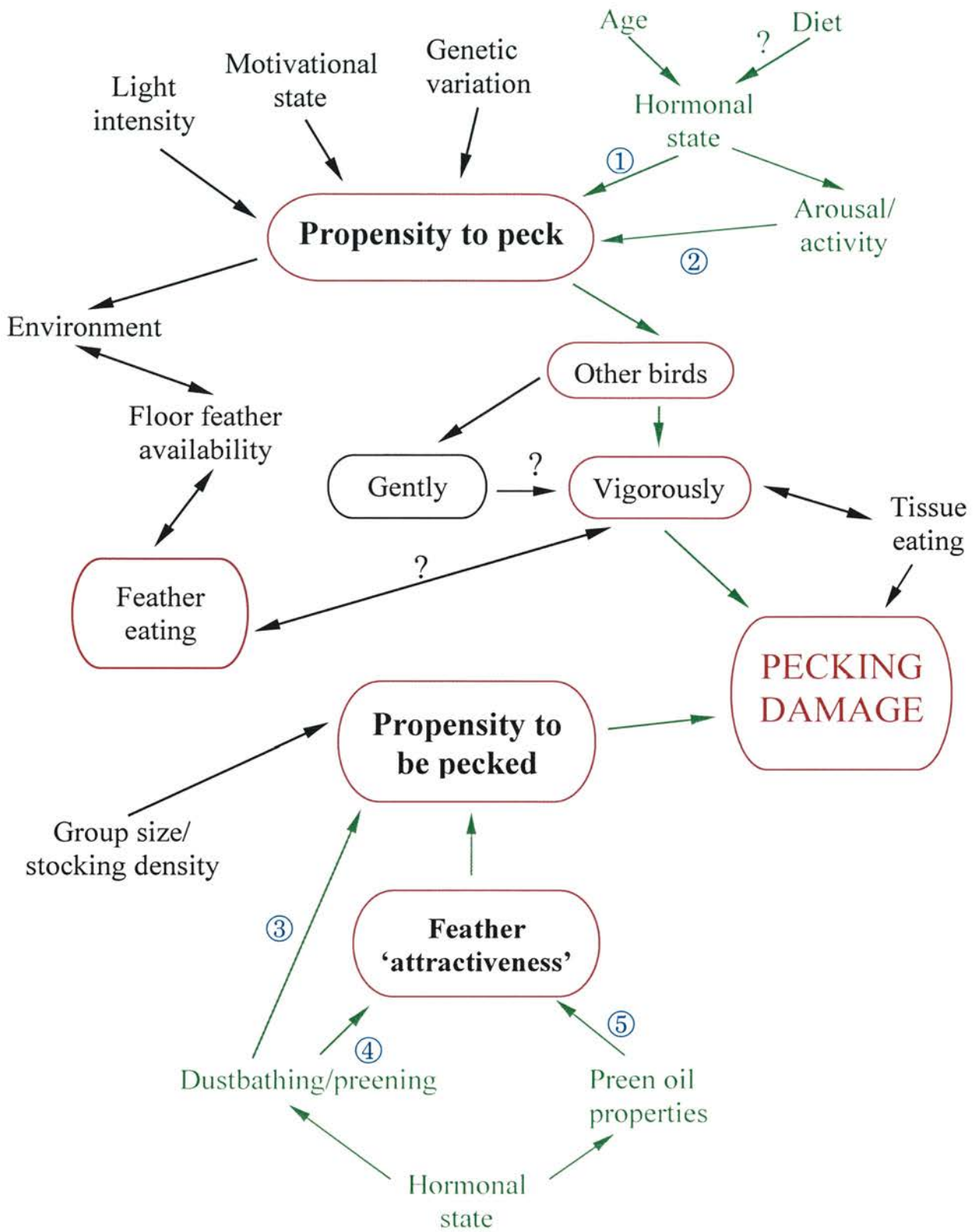


Figure 7.1 Proposed factors involved in the development of damaging pecking (adapted from C.J. Savory, unpublished). Pathways illustrating the possible involvement of gonadal hormones in the development of damaging pecking are shown in green.

Throughout the project, increased damaging pecking (related to changes in the number, type and targeting of bird to bird pecks) was observed at sexual maturity, and it is proposed that circulating concentrations of gonadal hormones act on propensity to peck directly (Figure 7.1, ①). Obviously, the coincidence in time of sexual maturation and increased damaging pecking provides circumstantial evidence for hormonal involvement. More direct evidence for an oestrogen receptor mediated mechanism for the control of pecking behaviour was obtained when treatment with tamoxifen suppressed pecking at a pecking device in adult hens (Chapter 4).

Damaging pecking is thought to be related to general activity level (Keeling and Jensen, 1995; Savory and Griffiths, 1997; Savory and Mann, 1997), which in turn has been linked to hormonal state in both domestic fowl (Horne and Wood-Gush, 1970) and mammals (Cushing et al., 1995). Thus, changes in hormonal state may also act on pecking behavior indirectly, by affecting levels of arousal/activity (Figure 7.1, ②).

Hormonal state could also influence the development of damaging pecking through specific behaviours. The results of Chapters 3 and 5 showed that the feather-related behaviours, preening and dustbathing, are exhibited to a greater extent at the onset of lay, a change which may be hormonally mediated. There is evidence to suggest that birds may attract pecking while performing these behaviours. Dustbathing birds attract pecks (Leonard et al., 1995; Savory and Mann, 1997) and Savory and Griffiths (1997) showed that birds which preened more received more feather pulls. Preening and dustbathing could affect propensity to be pecked (see Figure 7.1) through 2 mechanisms; directly, by drawing attention to the feathers by the act of the behaviour itself (Figure 7.1, ③), or indirectly by improving feather 'attractiveness' (Figure 7.1, ④). Conceivably, birds that preen more have more preen oil on their feathers, which might make them a more attractive target for pecking. In the flock as a whole, improvements in feather condition and/or increases in feather lipid content due to increased preening/dustbathing associated with sexual maturation could encourage damaging pecking at the onset of lay. The demonstrated association between feather pecking and feather eating and the preference for oiled feathers exhibited by individual hens (Chapter 6) supports the theory that preen oil has a role in the attractiveness of feathers to peck/eat.

Finally, the preen gland is known to be responsive to gonadal steroids, and it is possible that changes in circulating levels of sex steroids could act directly on its activity. Although little is known of the effects of gonadal steroids on the preen gland secretions of female domestic fowl, androgen receptors have been found in the preen glands of cockerels (Shanbhag and Sharp, 1996) and effects of testosterone on preen gland activity and chemical composition of secretions have been reported in male quail (Abalain et al., 1984; Amet et al., 1986). In addition, changes in the pheromonal properties of preen oil in female ducks during the mating season are known to be oestrogen mediated (Kolattukudy et al., 1987; Bohnet et al., 1991; Hiremath et al., 1992), and oestrogens can affect uropygial gland function in pigeons (Manna et al., 1983). Hormonally mediated changes in preen gland activity could influence feather attractiveness, by altering preen oil composition and thus the (possibly olfactory/gustatory) properties of oiled feathers (Figure 7.1, ⑤).

Throughout this project, problems were encountered with the unpredictability of damaging pecking. Although steps were taken to counteract this particular difficulty, such as the use of maximum numbers of birds (limited by cost and space) and the rearing of birds in conditions known to increase the likelihood of damaging pecking, studies were hindered either by a complete absence of damaging pecking or such severe outbreaks of pecking damage that pens had to be removed from the experiment with subsequent loss of data. These problems are likely to be attributable to the mathematical probability of the presence of 'peckers' (which are responsible for the majority of damaging pecks) in a given number of birds. Due to the negative exponential form of the frequency distribution observed in rates of vigorous pecking/pulling in individual birds (Savory and Griffiths, 1997; Wechsler et al., 1998), only a small proportion (<5%) of birds can be described as persistent peckers/pullers. Therefore, when birds are placed in small groups, pecking is seen in some pens (those containing persistent peckers/pullers) while no damage occurs in others. It should be noted however, that in large colonies kept in non-cage housing systems, a small number of such peckers/pullers is capable of inflicting serious and widespread pecking damage.

The layer pullets used in this project were maintained in conditions that simulated as closely as possible those encountered in commercial layer houses.

Whilst group sizes were clearly much smaller, stocking density, the thermal environment, dietary composition and husbandry practices were all those employed in the industry. The results of the present study provide information relevant to a commonly used commercial laying strain (ISA Brown), and therefore may have strategic implications. Experiments examining behavioural development showed that feather pecking in juveniles during the growing period (Chapter 3), and low floor feather availability (Chapter 6), are good indicators of more serious pecking problems at sexual maturity. Use of such an 'early warning system' could improve our ability to predict outbreaks of damaging pecking and help guide the use of pre-emptive measures such as reduced light levels and introduction of environmental enrichment devices. The results presented in Chapter 5 suggest a possible effect of dietary protein source on the development of damaging pecking, which may have implications for layer diets. More information is required, but dietary manipulation to provide the diet least likely to induce pecking problems has great potential as measure to reduce or even prevent damaging pecking as it is simple, flexible and can be applied to any type of production system. Supplementation of diets with tryptophan has been previously shown to reduce pecking damage (Savory et al., 1999), but this approach is prohibitively expensive for application to commercial egg production.

It is generally accepted that pecking problems occur to a greater extent in alternative, non-cage systems. With plans in place to phase out the keeping of laying hens in conventional battery cages in the European Union by 2012, there is an urgent need to improve our understanding of the multifactorial aetiology of feather pecking and cannibalism in order to help solve pecking problems. This project has identified some fundamental developmental changes and possible behavioural, hormonal and dietary mechanisms by which feather pecking can develop.

Reference list

- Abalain, J.H., Amet, Y., Daniel, J.Y., and Floch, H.H., 1984. Androgen regulation of secretions in the sebaceous-like uropygial gland of the male Japanese quail. *Journal of Endocrinology*, 103: 147-153.
- Abrahamsson, P. and Tauson, R., 1995. Aviary systems and conventional cages for laying hens. *Acta Agriculturae Scandinavica*, 45: 191-203.
- Abrahamsson, P., Tauson, R., and Appleby, M.C., 1995. Performance of four hybrids of laying hens in modified and conventional cages. *Acta Agriculturae Scandinavica*, 45: 286-296.
- Abrahamsson, P., Tauson, R., and Elwinger, K., 1996. Effects on production, health and egg quality of varying proportions of wheat and barley in diets for two hybrids of laying hens kept in different housing systems. *Acta Agriculturae Scandinavica*, 46: 173-182.
- Akiba, Y., Jensen, L.S., Barb, C.R., and 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. and Elwinger, K., 1987a. 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. and Elwinger, K., 1987b. 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. and Combs, G.F., 1981. Effects on feed palatability of ingredients believed to contain unidentified growth factors for poultry. *Poultry Science*, 60: 215-224.
- Allee, W.C. and Collias, N., 1940. The influence of oestradiol on the social organisation of flocks of hens. *Endocrinology*, 27: 87-94.
- Allee, W.C., Collias, N., and Lutherman, C.Z., 1939. Modification of the social order in flocks of hens by the injection of testosterone propionate. *Physiological Zoology*, 14: 415-440.
- Allen, J. and 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., Weaver, G., Aakhusallen, S., and Bates, F., 1981. Effects of dietary zearalenone on finishing broiler-chickens and young turkey poults. *Poultry Science*, 60: 124-131.
- Ambrosen, T. and 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.
- Amet, Y., Abalain, J.H., Distefano, S., Daniel, J.Y., Tea, K., Floch, H.H., and Robel, P., 1986. Androgen regulation of the androgen receptor of the quail uropygial gland. *Journal of Endocrinology*, 109: 299-306.
- Andrew, R.J. and Jones, R.B., 1992. Increased distractability in capons: an adult parallel to androgen-induced effects in the domestic chick. *Behavioural Processes*, 26: 201-210.
- Annisson, E.F., 1983. Lipid metabolism. In: Freeman, B.M. (Editors), *Physiology and Biochemistry of the domestic fowl*, Volume 5. Academic Press, London.
- Appleby, M.C., 1993. Should cages for laying hens be banned or modified? *Animal Welfare*, 2: 67-80.
- Appleby, M.C., Hogarth, G.S., Anderson, J.A., Hughes, B.O., and Whittemore, C.T., 1988. Performance of a deep litter system for egg production. *British Poultry Science*, 29: 735-751.
- Appleby, M.C., Hughes, B.O., and Elson, H.A., 1992. *Poultry Production Systems: Behaviour, Management and Welfare*. CAB International, Wallingford, UK.
- Appleby, M.C., Smith, S.F., and Hughes, B.O., 1993. Nesting, dust bathing and perching by laying hens in cages: effects of design on behaviour and welfare. *British Poultry Science*, 34: 835-847.
- Atteh, J.O. and 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.
- Axelsson, M., Sjøvall, J., Gustafsson, B.E., and 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.
- Bacon, W.L. and Touchbarn, S.P., 1976. Effect of light intensity on confinement rearing of male turkeys. *Poultry Science*, 55: 999-1007.
- Bar, A., Vax, E., Hunziker, W., Halevy, O., and Striem, S., 1996. Role of gonadal hormones in gene expression of calbindin (M(r)28,000) in the laying hen. *General and Comparative Endocrinology*, 103: 115-122.
- Bearse, G.E., Berg, L.R., McClary, C.F., and Miller, V.L., 1949. The effect of pelleting chicken rations on the incidence of cannibalism. *Poultry Science*, 28: 756.

- Bearse, G.E., Miller, V.L., and McClary, C.F., 1940. The cannibalism preventing properties of the fiber fraction of oat hulls. *Poultry Science*, 19: 210-215.
- Bedrak, E., Harvey, S., and Chadwick, A., 1981. Concentrations of pituitary, gonadal adrenal hormones in serum of laying and broody white rock hens (*Gallus domesticus*). *Journal of Endocrinology*, 91: 197-204.
- Beguín, D.P. and Kincaid, R.L., 1984. 3-Hydroxy-3-methyl-glutaryl coenzyme-a reductase-activity in chicks fed coumestrol, a phytoestrogen. *Poultry Science*, 63: 686-690.
- Bell, D.D., Adams, C.J., and Gvoryahu, G., 1998. Environmental enrichment devices for caged laying hens. *Journal of Applied Poultry Research*, 7: 19-26.
- Bessei, W., 1978. Der Einflub einer Na-armen ration auf die laufaktivitat von legehennen. *Archiv fur Geflugelkunde*, 42: 246-248.
- Bessei, W., Reiter, K., and Schwarzenberg, A., 1997. Measuring pecking towards a bunch of feathers in individually housed hens as a means to select against feather pecking. In: Proceedings 5th European Symposium on Poultry Welfare, 74-76. Wageningen, The Netherlands.
- Bhagwat, A.L. and Craig, J.V., 1977. Selecting for age at first egg: effects on social dominance. *Poultry Science*, 56: 361-363.
- Bilcik, B. and 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., and Coward, A., 1998. Phyto-oestrogens: Where are we now? *British Journal of Nutrition*, 79: 393-406.
- Blokhuis, H.J., 1986. Feather-pecking in poultry - its relation with ground-pecking. *Applied Animal Behaviour Science*, 16: 63-67.
- Blokhuis, H.J., 1989. The effect of a sudden change in floor type on pecking behavior in chicks. *Applied Animal Behaviour Science*, 22: 65-73.
- Blokhuis, H.J., 1991. Effects of rearing conditions on feather pecking in laying hens. *Applied Animal Behaviour Science*, 30: 186-186.
- Blokhuis, H.J. and Arkes, J.G., 1984. Some observations on the development of feather pecking in poultry. *Applied Animal Behaviour Science*, 12: 145-157.
- Blokhuis, H.J. and Beuving, G., 1993. Feather pecking and other characteristics in two lines of laying hens. In: Savory, C. J. and Hughes, B. O. (Editors), Proceedings of the 4th European Symposium on Poultry Welfare, 145-157. Universities Federation for Animal Welfare, Potters Bar.

- Blokhuis, H.J. and 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. and Vanderhaar, J.W., 1992. Effects of pecking incentives during rearing on feather pecking of laying hens. *British Poultry Science*, 33: 17-24.
- Blokhuis, H.J. and Wiepkema, P.R., 1998. Studies of feather pecking in poultry. *Veterinary Quarterly*, 20: 6-9.
- Bohnet, S., Rogers, L., Sasaki, G.C., and Kolattukudy, P.E., 1991. Estradiol induces proliferation of peroxisome-like microbodies and the production of 3-hydroxy fatty-acid diesters, the female pheromones, in the uropygial glands of male and female mallards. *Journal of Biological Chemistry*, 266: 9795-9804.
- Bolliger, A. and Varga, D., 1961. Feather Lipids. *Nature*, 4781: 1125.
- Bolton, W. and Blair, R., 1974. Poultry Nutrition. HMSO, London.
- Boshouwers, F.M.G. and Nicaise, E., 1987. Physical activity and energy expenditure of laying hens as affected by light intensity. *British Poultry Science*, 28: 155-163.
- Boshouwers, F.M.G. and Nicaise, E., 1993. Artificial light sources and their influence on physical activity and energy expenditure of laying hens. *British Poultry Science*, 34: 11-19.
- Bradshaw, M.S., Tsai, S.Y., Leng, X.H., Dobson, A.D.W., Coneely, O.M., OMalley, B.W., and Tsai, M.J., 1991. Studies on the mechanism of functional cooperativity between progesterone and oestrogen-receptors. *Journal of Biological Chemistry*, 25: 16684-16690.
- Breward, J. and Gentle, M.J., 1985. Neuroma formation and abnormal afferent nerve discharges after partial beak amputation (beak trimming) in poultry. *Experientia*, 41: 1132-1134.
- Cain, J.R., Weber, J.M., Lockamy, T.A., and Creger, C.R., 1984. Grower diets and bird density effects on growth and cannibalism in Ring-Necked Pheasants. *Poultry Science*, 63: 450-457.
- Calet, C., 1965. The relative value of pellets versus mash and grain in poultry nutrition. *Worlds Poultry Science Journal*, 21: 23-52.
- Cantor, A.H. and 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., and Common, R.H., 1965. Urinary conversion products of biochanin A and formononetin in the fowl. *Biochemica Et Biophysica Acta*, 111: 349-357.

- Channing, C.E., Hocking, P.M., and Jones, R.B., 1998. Feather pecking in adult laying hens: can it be associated with pecking at inanimate objects? *British Poultry Science*, 39: S15-S16.
- Cook, R.E. and Warnik, A.C., 1962. The effects of progesterone and progestational compounds upon the production of laying pullets. *Poultry Science*, 41: 1545-1550.
- Craig, J.V. and Muir, W.M., 1993. Selection for reduction of beak-inflicted injuries among caged laying hens. *Poultry Science*, 72: 411-420.
- Curtis, P.E. and Marsh, N.W.A., 1992. Cannibalism in laying hens. *Veterinary Record*, 424.
- Cushing, B.S., Marhenke, S., and McClure, P.A., 1995. Estradiol concentration and the regulation of locomotor-activity. *Physiology & Behavior*, 58: 953-957.
- Cuthbertson, G. J., 1978. Ph.D. Thesis, University of Edinburgh.
- Cuthbertson, G.J., 1980. Genetic variation in feather pecking behaviour. *British Poultry Science*, 21: 447-450.
- Deag, J.M., 1994. KEYBEHAVIOUR, a program for recording behavioural data on the DIP Pocket PC and Atari Portfolio computer. Version TPPF3.4b.
- Dickerson, G. and Kashyap, T., 1961. Heritable variation in pecking behaviour of chickens. *Poultry Science*, 40: 1394-1395.
- Duncan, I.J.H., Slee, G.S., Seawright, E., and Breward, J., 1989. Behavioural consequences of partial beak amputation (Beak trimming) in poultry. *British Poultry Science*, 30: 479-488.
- Dziuk, P.J. and Cook, B., 1966. Passage of steroids through silicone rubber. *Endocrinology*, 78: 208-211.
- Epple, A. and Stetson, M.H., 1980. Avian Endocrinology. Academic Press, New York.
- Etches, R.J., 1996. Reproduction in poultry. CAB International, Wallingford, UK.
- Etches, R.J. and Cunningham, F.J., 1976. The interrelationship between progesterone and luteinizing hormone during the ovulation cycle of the hen (*Gallus domesticus*). *Journal of Endocrinology*, 71: 51-58.
- Etches, R.J. and Cunningham, F.J., 1977. The plasma concentrations of testosterone and LH during the ovulation cycle of the hen (*Gallus domesticus*). *Acta Endocrinologica*, 84: 357-366.
- Farm Animal Welfare Council, 1997. Report on the welfare of laying hens (MAFF, Tolworth).

- Fennell, M.J. and Scanes, C.G., 1992. Inhibition of growth in chickens by testosterone, 5 α -dihydrotestosterone, and 19-nortestosterone. *Poultry Science*, 71: 357-366.
- Fraile, I.G., Pfaff, D.W., and McEwen, B.S., 1987. Progesterin receptors with and without estrogen induction in male and female hamster brain. *Neuroendocrinology*, 45: 487-491.
- Gao, Y. and Short, R.V., 1994a. Fertility control in laboratory rats and mice after feeding with the antigestagen RU486. *Journal of Reproduction and Fertility*, 101: 477-481.
- Gao, Y. and Short, R.V., 1994b. Fertility control in wild mice after feeding with RU486 or methyl testosterone. *Journal of Reproduction and Fertility*, 101: 483-487.
- Gentle, M.J., Waddington, D., Hunter, L.N., and Jones, R.B., 1990. Behavioural evidence for persistent pain following partial beak amputation in chickens. *Applied Animal Behaviour Science*, 27: 149-157.
- Gordon, D.A., Shelness, G.S., Nicosia, M., and Williams, D.L., 1988. Estrogen-induced destabilization of yolk precursor protein mRNAs in avian liver. *The Journal of Biological Chemistry*, 263: 2625-2631.
- Gunnarsson, S., Keeling, L., and Svedberg, J., 1999. Effect of rearing factors on the prevalence of floor eggs, cloacal cannibalism and feather pecking in commercial flocks of loose housed laying hens. *British Poultry Science*, 40: 12-18.
- Gvoryahu, G., Ararat, E., Asaf, E., Lev, M., Weller, J.I., Robinzon, B., and Snapir, N., 1994. An enrichment object that reduces aggressiveness and mortality in caged laying hens. *Physiology & Behavior*, 55: 313-316.
- Haahti, E.O.A. and Fales, H.M., 1967. The uropygiols: identification of the unsaponifiable constituent of a diester wax from chicken preen glands. *Journal of Lipid Research*, 8: 131-137.
- Hagan, R.C., Leszczynski, D.E., and Kummerow, F.A., 1984. Comparative plasma lipid response of pullets and laying hens to estradiol and progesterone. *Toxicology and Applied Pharmacology*, 76: 483-489.
- Hagen, D.R. and Dzuik, P.J., 1985. The effect of exogenous testosterone on homospermic and heterospermic fertility in the cock. *Biology of Reproduction*, 32: 1080-1086.
- Hermier, D., Catheline, D., and Legrand, P., 1996. Relationship between hepatic fatty acid desaturation and lipid secretion in the estrogenized chicken. *Comparative Biochemistry and Physiology*, 115: 259-264.

- Heywang, B.W. and Morgan, R.B., 1944. A comparison of a pelleted and unpelleted all-mash diet for growing chickens. *Poultry Science*, 23: 16-20.
- Hiremath, L.S., Kessler, P.M., Sasaki, G.C., and Kolattukudy, P.E., 1992. Estrogen induction of alcohol-dehydrogenase in the uropygial gland of mallard ducks. *European Journal of Biochemistry*, 203: 449-457.
- Hiura, Y., 1990. Studies on the relationship between concentration of yolk precursor in blood, growth of follicle and structure of yolk spheres in laying hens treated with antioestrogen or oestrogen. *Japanese Poultry Science*, 27: 191-199.
- Hoffmeyer, I., 1969. Feather pecking in pheasants - an ethological approach to the problem. *Danish Review of Game Biology*, 6: 1-36.
- Hollmann, P., 1997. Behaviour disorders in psittacines: 1. Signs and causes. *Tierärztliche Praxis*, 25: 233-240.
- Horne, A.R. and Wood-Gush, D.G.M., 1970. Exploratory activity following oestrogen treatment in ovariectomised brown leghorn females. *Rev Comportement Animal*, 4: 46-50.
- Huber-Eicher, B. and Wechsler, B., 1997. Feather pecking in domestic chicks: its relation to dustbathing and foraging. *Animal Behaviour*, 54: 757-768.
- Huber-Eicher, B. and Wechsler, B., 1998. The effect of quality and availability of foraging materials on feather pecking in laying hen chicks. *Animal Behaviour*, 55: 861-873.
- Hughes, B.O., 1973. The effect of implanted gonadal hormones on feather pecking and cannibalism in pullets. *British Poultry Science*, 14: 341-348.
- Hughes, B.O., 1980. Feather damage in hens caged individually. *British Poultry Science*, 21: 149-154.
- Hughes, B.O., 1985. Feather loss as a problem: how does it occur? In: Proceedings, 2nd European Seminar on Poultry Welfare, 177-188. World's Poultry Science Association.
- Hughes, B.O. and Black, A.J., 1974. The effect of environmental factors on activity, selected behaviour patterns and 'fear' of fowls in cages and pens. *British Poultry Science*, 15: 375-380.
- Hughes, B.O. and Black, A.J., 1976. Battery cage shape: its effect on diurnal feeding pattern, egg shell cracking and feather pecking. *British Poultry Science*, 17: 327-336.
- Hughes, B.O. and Duncan, I.J.H., 1972. The influence of strain and environmental factors upon feather pecking and cannibalism in fowls. *British Poultry Science*, 13: 525-547.

- Hughes, B.O. and Mitchie, W., 1982. Plumage loss in medium-bodied hybrid laying hens: the effect of beak trimming and cage design. *British Poultry Science*, 13: 59-64.
- Hughes, B.O. and Whitehead, C.C., 1974. Sodium deprivation, feather pecking and activity in laying hens. *British Poultry Science*, 15: 435-439.
- Hutson, L.R., 1978. Pica in eight-week-old chicken broilers. *Veterinary Record*, 103: 186-187.
- Itoh, H., Kono, T., Kuwayama, T., and Ichinoe, K., 1988. The relationship between plasma concentrations of progesterone, estradiol and testosterone and the ages of first egg laying in maturing pullets. *Japanese Poultry Science*, 25: 86-92.
- Jacoby, S., Arnon, E., Snapir, N., and Robinzon, B., 1995. Effects of estradiol and tamoxifen on feeding, fattiness and some endocrine criteria in hypothalamic obese hens. *Pharmacology Biochemistry and Behaviour*, 50: 55-63.
- Jacoby, S., Snapir, N., Rozenboim, I., Arnon, R., Meidan, R., and Robinzon, B., 1992. Tamoxifen advances puberty in the white leghorn hen. *British Poultry Science*, 33: 101-111.
- Jacob, J., Balthazart, J., and Schoffeniels, E., 1979. Sex differences in the chemical composition of uropygial gland waxes in domestic ducks. *Biochemical Systematics and Ecology*, 7: 149-153.
- Jacob, J. and Ziswiler, V., 1982. The uropygial gland. In: King, J.R. and Parkes, K.C. (Editors), *Avian Biology VI*. Academic Press, New York.
- Jarvinen, A., Nykanen, S., and Paasiniemi, L., 1999. Absorption and bioavailability of oestradiol from a gel, a patch and a tablet. *Maturitas*, 32: 103-113.
- Jensen, L.S., Merrill, L.H., Reddy, C.V., and McGinnus, J., 1962. Observations on eating patterns and rate of food passage of birds fed pelleted and unpelleted diets. *Poultry Science*, 41: 1414-1419.
- Johnsen, P.F., Vestergaard, K.S., and NorgaardNielsen, G., 1998. Influence of early rearing conditions on the development of feather pecking and cannibalism in domestic fowl. *Applied Animal Behaviour Science*, 60: 25-41.
- Johnson, A.L., 1983. Progesterone-induced delay of the onset of lay in the prepubertal pullet. *Poultry Science*, 62: 2474-2478.
- Johnson, A.L. and van Tienhoven, A., 1981. Pharmacokinetics of estradiol-17 β in the laying hen. *Poultry Science*, 60: 2720-2723.
- Jones, R.B., 1987. Social and environmental aspects of fear in the domestic fowl. In: Zayan, R. and Duncan, I.J.H. (Editors), *Cognitive aspects of social behaviour in the domestic fowl*. Elsevier, Amsterdam.

- Jones, R.B., 1996. Fear and adaptability in poultry: insights, implications and imperatives. *Worlds Poultry Science Journal* , 52: 131-174.
- Jones, R.B. and Andrew, R.J., 1992. Responses of adult domestic cocks and capons to novel and alarming stimuli. *Behavioural Processes*, 26: 189-200.
- Jones, R.B., Blokhuis, H.J., and Beuving, G., 1995. Open-field and tonic immobility responses in domestic chicks of two genetic lines differing in their propensity to feather peck. *British Poultry Science*, 36: 525-530.
- Jones, R.B. and Carmichael, N.L., 1998. Pecking at string by individually caged, adult laying hens: colour preferences and their stability. *Applied Animal Behaviour Science*, 60: 11-23.
- Jones, R.B. and Faure, J.M., 1982. Domestic chicks prefer familiar soiled substrate in an otherwise novel environment. *IRCS Medical Science*, 10: 847.
- Jones, R.B. and Gentle, M.J., 1985. Olfaction and behavioural modification in domestic chicks (*Gallus Domesticus*). *Physiology & Behavior*, 34: 917-924.
- Jones, R.B. and Roper, T.J., 1997. Olfaction in the domestic fowl: a critical review. *Physiology & Behavior*, 62: 1009-1018.
- Kamiyoshi, M., Kawashima, M., and Tanaka, K., 1992. Estrogen primes cultured granulosa cells of the hen for progesterone production in response to LH. *Japanese Poultry Science*, 29: 91-97.
- Kawashima, M., Kamiyoshi, M., and Tanaka, K., 1979. Effects of progesterone, estradiol and testosterone on cytoplasmic progesterone receptor concentrations in the hen hypothalamus and pituitary. *Biology of Reproduction*, 21: 639-646.
- Kawashima, M., Ukai, A., Kamiyoshi, M., and Tanaka, K., 1992. Enhancement of progesterone-receptor bindings by estradiol in pituitary-cells of the hen. *Poultry Science*, 71: 352-356.
- Keeling, L., 1994. Feather pecking - who in the group does it, how often and under what circumstances? In: Proceedings of the 9th European Poultry Conference, 288-299.
- Keeling, L., 1995. Feather pecking and cannibalism in layers. *Poultry International*, 34: 46-50.
- Keeling, L. and Wilhelmson, M., 1997. Selection based on direct observations of feather pecking behaviour in adult laying hens. In: Proceedings 5th European Symposium on Poultry Welfare, 74-76. Wageningen, The Netherlands.
- Keeling, L.J. and Jensen, P., 1995. Do feather pecking and cannibalistic hens have different personalities? *Applied Animal Behaviour Science*, 44: 257-281.

- Khalafalla, M.K., Bessei, W., and Schwarzenberg, A., 1988. Effect of mineral salts in drinking water on the domestic chicken performance - literature review. *Archiv fur Geflugelkunde*, 62: 26-32.
- Kivimae, A., 1976. The influence of floor area per hen and the number of hens per cage on the performance and behaviour of laying hens. *Archiv fur Geflugelkunde*, 40: 202-205.
- Kjaer, J.B., 1995. Light intensity affects physical activity and feather pecking in chickens. In: Proceedings of the 1st North American Symposium on Poultry Welfare.
- Kjaer, J.B., 1997. Effect of light intensity on growth, feed intake and feather pecking behaviour in beak trimmed and bitted pheasant chickens (*Phasianus colchicus*). *Archiv fur Geflugelkunde*, 61: 167-171.
- Kjaer, J.B. and Sorensen, P., 1997. Feather pecking behaviour in White Leghorns, a genetic study. *British Poultry Science*, 38: 333-341.
- Kjaer, J.B. and Vestergaard, K.S., 1999. Development of feather pecking in relation to light intensity. *Applied Animal Behaviour Science*, 62: 243-254.
- Knight, D.C. and Eden, J.A., 1995. Phytoestrogens - a short review. *Maturitas*, 22: 167-175.
- Kolattukudy, P.E., Bohnet, S., and Rogers, L., 1987. Diesters of 3-hydroxy fatty acids produced by the uropygial glands of female mallards uniquely during the mating season. *Journal of Lipid Research*, 28: 582-588.
- Kono, T., Kuwayama, T., Itoh, H., and Inchinoe, K., 1999. Nesting behaviour and changes in plasma concentrations of progesterone, testosterone and estradiol in the native japanese breed of chicken, *Gifujidori (Gallus domesticus)*. *Japanese Poultry Science*, 22: 64-72.
- Korte, S.M., Beuving, G., Ruesink, W., and Blokhuis, H.J., 1997. Plasma catecholamine and corticosterone levels during manual restraint in chicks from a high and low feather pecking line of laying hens. *Physiology & Behavior*, 62: 437-441.
- Korte, S.M., Ruesink, W., and Blokhuis, H.J., 1999. Heart rate variability during manual restraint in chicks from high- and low-feather pecking lines of laying hens. *Physiology & Behavior*, 65: 649-652.
- Lanson, R.K. and Smyth, J.R., 1955. Pellets vs. mash plus pellets vs. mash for broiler feeding. *Poultry Science*, 34: 234-235.
- Laugier, C., Fanidi, A., Dufrene, L., Fayard, J., and Pageaux, J., 1991. Dissociated effects of tamoxifen on growth and progesterone receptor induction in quail oviduct. *General and Comparative Endocrinology*, 83: 439-446.

- Lazier, C.B., 1987. Interactions of tamoxifen in chicken. *Journal of Steroid Biochemistry*, 27: 877-882.
- Leonard, M.L., Horn, A.G., and Fairfull, R.W., 1995. Correlates and consequences of allopecking in white-leghorn chickens. *Applied Animal Behaviour Science*, 43: 17-26.
- Leopold, A.S., Erwin, M., Oh, J., and Browning, B., 1976. Phytoestrogens: adverse effects on reproduction in California quail. *Science*, 191: 98-100.
- Lewis, P.D. and Morris, T.R., 1998. Responses of domestic poultry to various light sources. *Worlds Poultry Science Journal*, 54: 7-25.
- Lifchez, A. and Scommegna, A., 1970. Diffusion of progestogens through silastic rubber implants. *Fertility and Sterility*, 21: 426-430.
- Lindberg, A.C. and Nicol, C.J., 1994. An evaluation of the effect of operant feeders on welfare of hens maintained on litter. *Applied Animal Behaviour Science*, 41: 211-227.
- Lobo, R.A. and Cassidenti, D.L., 1992. Pharmacokinetics of oral-17 beta-estradiol. *Journal of Reproductive Medicine*, 37: 77-84.
- Malik, D.D. and Aggarwal, C.K., 1970. Effects of oral administration of synthetic sex hormones to White Leghorn cockerels. *Indian Journal of Poultry Science*, 5: 41-45.
- Manna, C.K., Deadhikari, H., and Bhattacharya, S.P., 1983. Action of estriol on the uropygial gland activity in pigeon. *Zeitschrift Fur Mikroskopisch-Anatomische Forschung*, 97: 386-392.
- Martin, G., 1989. Federpickhaufigkeit in Abhängigkeit von Draht- und Einstreuboden sowie von der Lichtintensität. *Kuratorium für Technik und Bauwesen in der Landwirtschaft Schrift*, 342: 108-133.
- Martin, P. and Bateson, P., 1986. Measuring behaviour: an introductory guide. Cambridge University Press, Cambridge.
- Mashaly, M.M., Proudman, J.A., and Wentworth, B.C., 1979. Reproductive performance of turkey hens as influenced by hormone implants. *British Poultry Science*, 20: 19-26.
- Maurice, D.V., Jensen, L.S., and 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.
- Miller, M.W. and Bearnse, G.E., 1937. The cannibalism preventing properties of oats. *Poultry Science*, 16: 314-321.

- Miller, M.W. and Bearnse, G.E., 1938. The cannibalism preventing properties of oat hulls. *Poultry Science*, 17: 466-471.
- Mills, A.D. and Faure, J.M., 1990. Panic and hysteria in domestic fowl. In: Zayan, R. and Dantzer, R. (Editors), *Social stress in domestic animals*. Kluwer Academic Publishers, Dordrecht-Boston-London.
- Mitchell, M.A. and Carlisle, A.J., 1991. Plasma zinc as an index of vitellogenin production and reproductive status in the domestic fowl. *Comparative Biochemistry and Physiology*, 100A: 719-724.
- Mohsin, M. and 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.
- Moinard, C., Morisse, J.P., and Faure, J.M., 1998. Effect of cage area, cage height and perches on feather condition, bone breakage and mortality of laying hens. *British Poultry Science*, 39: 198-202.
- Mundheim, H. and 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.
- Murphy, L.B., 1977. Responses of domestic fowl to novel food and novel objects. *Applied Animal Ethology*, 3: 335-349.
- Nahoul, K., Dehennin, L., Jondet, M., and Roger, M., 1993. Profiles of plasma estrogens, progesterone and their metabolites after oral or vaginal administration of estradiol or progesterone. *Maturitas*, 16: 185-202.
- Neal, W.M., 1956. Cannibalism, pick-outs and methionine. *Poultry Science*, 35: 10-13.
- Newbold, R.R., Bullock, B.C., and McLachlan, J.A., 1990. Uterine adenocarcinoma in mice following developmental treatment with oestrogen: a model for hormonal carcinogenesis. *Cancer Research*, 50: 7677-7681.
- Noddegard, F. and Kennaway, D.J., 1999. A method for achieving physiological plasma levels of melatonin in the chicken by oral administration. *Journal of Pineal Research*, 27: 129-138.
- Norgaard-Nielsen, G., 1997. Dustbathing and feather pecking in domestic chickens reared with and without access to sand. *Applied Animal Behaviour Science*, 52: 99-108.
- Norgaard-Nielsen, G., Vestergaard, K.S., and Simonsen, H.B., 1993. Effects of rearing experience and stimulus enrichment on feather damage in laying hens. *Applied Animal Behaviour Science*, 38: 345-352.

- Oden, K., Vestergaard, K.S., and Algers, B., 1999. Agonistic behaviour and feather pecking in single-sexed and mixed groups of laying hens. *Applied Animal Behaviour Science*, 62: 219-231.
- Ouart, M.D. and Adams, A.W., 1982. Effects of cage design and bird density on layers .1. Productivity, feathering, and nervousness. *Poultry Science*, 61: 1606-1613.
- Pardue, S.L., Brake, J., Seib, P.A., and Wang, X.Y., 1993. Relative bioavailability of L-ascorbyl-2-polyphosphate in broiler chickens. *Poultry Science*, 72: 1330-1338.
- Peguri, A. and Coon, C., 1993. Effect of feather cover and temperature on layer performance. *Poultry Science*, 72: 1318-1329.
- Pelissero, C., Cuisset, B., and 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., and 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.
- Piersma, T. and van Eerden, M.R., 1989. Feather eating in Great Crested Grebes *Podiceps cristatus*: a unique solution to the problems of debris and gastric parasites in fish eating birds. *Ibis*, 131: 477-486.
- Rawles, M.E., 1960. The integumentary system. In: Marshall, A.J. (Editors), *Biology and comparative physiology of birds*. Academic Press, New York, London.
- Richards, S.A., 1977. The influence of loss of plumage on temperature regulation in laying hens. *J.agric.Sci.Camb.*, 89: 393-398.
- RichardYris, M.A., Guemene, D., Lea, R.W., Sharp, P.J., Bedecarrats, G., Foraste, M., and Wauters, A.M., 1998. Behaviour and hormone concentrations in nest deprived and renesting hens. *British Poultry Science*, 39: 309-317.
- Roden, C. and Wechsler, B., 1998. A comparison of the behaviour of domestic chicks reared with or without a hen in enriched pens. *Applied Animal Behaviour Science*, 55: 317-326.
- Saito, N., Tsuzuki, N., and Shimada, K., 1986. Prolonged inhibitory effect of progesterone on ovulation in the domestic hen (*Gallus domesticus*). *Japanese Poultry Science*, 23: 211-221.
- Samson, J., 1996. Behavioural problems of farmed ostriches in Canada. *Canadian Veterinary Journal*, 37: 412-414.

- Sanctuary, W.C., 1954. Vicious habits and cannibalism. *Worlds Poultry Science Journal*, 10: 356.
- Sanotra, G.S., Vestergaard, K.S., Agger, J.F., and Lawson, L.G., 1995. The relative preferences for feathers, straw, wood-shavings and sand for dustbathing, pecking and scratching in domestic chicks. *Applied Animal Behaviour Science*, 43: 263-277.
- Sasaki, H., Coffey, P., Villegas-Perez, M.P., Vidal-Sanz, M., Young, M.J., Lund, R.D., and Fukuda, Y., 1996. Light induced EEG desynchronization and behavioural arousal in rats with restored retinocollicular projection by peripheral nerve graft. *Neuroscience Letters*, 218: 45-48.
- Savory, C.J., 1995. Feather pecking and cannibalism. *Worlds Poultry Science Journal*, 51: 215-219.
- Savory, C.J. and Griffiths, J.D., 1997. Individual variation in rates of giving and receiving feather pecks in bantams, and some behavioural correlates. In: 5th European Symposium on Poultry Welfare, Wageningen, The Netherlands.
- Savory, C.J. and Hetherington, J.D., 1997. Effects of plastic anti-pecking devices on food intake and behaviour of laying hens fed on pellets or mash. *British Poultry Science*, 38: 125-131.
- Savory, C.J. and 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. and Mann, J.S., 1999. Feather pecking in groups of growing bantams in relation to floor litter substrate and plumage colour. *British Poultry Science*, In Press.
- Savory, C.J., Mann, J.S., and McLeod, 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.
- Schumaier, G., Harrison, P.C., and McGinnis, J., 1968. Effect of coloured fluorescent light on growth, cannibalism, and subsequent egg production of single comb white leghorn pullets. *Poultry Science*, 47: 1599-1602.
- Senior, B.E., 1974a. 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.
- Senior, B.E., 1974b. Changes in the concentrations of oestrone and oestradiol in the peripheral plasma of the domestic hen during the ovulatory cycle. *Acta Endocrinologica*, 77: 588-596.

- Shanbhag, B.A. and Sharp, P.J., 1996. Immunocytochemical localisation of androgen receptor in the comb, uropygial gland, testis, and epididymis in the domestic chicken. *General and Comparative Endocrinology*, 101: 76-82.
- 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, and L.S., 1987. Non-steroidal oestrogens of dietary origin: activity, distribution and mechanism of action. *Israeli Journal of Veterinary Medicine*, 43: 192-197.
- Shenfield, G.M. and Griffin, J.M., 1991. Clinical pharmacokinetics of contraceptive steroids - an update. *Clinical Pharmacokinetics*, 20: 15-37.
- Sherwin, C.M., 1993. Pecking behaviour of laying hens provided with a simple motorised environmental enrichment device. *British Poultry Science*, 34: 235-240.
- Siegel, S., 1956. Nonparametric statistics for the behavioural sciences. McGraw-Hill, New York.
- Simmons, K.E.L., 1956. Feather eating and pellet-formation in the Great Crested Grebe. *British Birds*, 49: 432-435.
- Simonsen, H.B., Vestergaard, K.S., and Willeberg, P., 1980. Effect of floor type and density on the integument of egg-layers. *Poultry Science*, 59: 2202-2206.
- Siopes, T.D., Timmons, M.B., Baughman, G.R., and Parkhurst, C.R., 1984. The effects of light intensity on turkey poult performance, eye morphology and adrenal weight. *Poultry Science*, 63: 904-909.
- Stewart, J.S., 1994. Ostrich behaviour and behavioural problems. *Proceedings of the Association of Avian Veterinarians*, 104-109.
- Sutherland, R.L., 1981. Estrogen antagonists in chick oviduct: antagonist activity of eight synthetic triphenylethylene derivatives and their interaction with cytoplasmic and nuclear estrogen receptors. *Endocrinology*, 109: 2061-2072.
- Tauson, R., 1998. Health and production in improved cage designs. *Poultry Science*, 77: 1820-1827.
- Tsang, C.P.W. and Grunder, A., 1984. Production, clearance rates and metabolic fate of oestradiol-17 β plasma of the laying hen. *Steroids*, 43: 71-84.
- Tullett, S.G., Macleod, M.G., and Jewitt, T.R., 1980. The effects of partial defeathering on energy metabolism in the laying fowl. *British Poultry Science*, 21: 241-245.

- Turnhill, L. and Feltwell, R., 1952. The complete poultryman. Faber and Faber, London.
- Vestergaard, K.S., Kruijt, J.P., and Hogan, J.A., 1993. Feather pecking and chronic fear in groups of red junglefowl: their relations to dustbathing, rearing environment and social status. *Animal Behaviour*, 45: 1127-1170.
- Vestergaard, K.S. and Lisborg, L., 1993. A model of feather pecking development which relates to dustbathing in the fowl. *Behaviour*, 126: 291-308.
- Vestergaard, K.S., Skadhauge, E., and Lawson, L.G., 1997. The stress of not being able to perform dustbathing in laying hens. *Physiology & Behavior*, 62: 413-419.
- Webb, R., Baxter, G., Nordblom, G.D., McBride, D., and 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.
- Wechsler, B. and Huber-Eicher, B., 1998. The effect of foraging material and perch height on feather pecking and feather damage in laying hens. *Applied Animal Behaviour Science*, 58: 131-141.
- Wechsler, B., HuberEicher, B., and Nash, D.R., 1998. Feather pecking in growers: a study with individually marked birds. *British Poultry Science*, 39: 178-185.
- Wells, R.G., 1971. A comparison of red and white light and low and high protein regimes for growing pullets. *British Poultry Science*, 12: 313-325.
- Widowski, T.M., Keeling, L., and Duncan, I.J.H., 1992. The preferences of hens for compact fluorescent over incandescent lighting. *Canadian Journal of Animal Science*, 72: 203-211.
- Williams, J.B. and Sharp, P.J., 1977. A comparison of plasma progesterone and luteinizing hormone in growing hens from eight weeks of age to sexual maturity. *Journal of Endocrinology*, 75: 447-448.
- Willimon, C.P. and Morgan, C.L., 1953. The effect of minor nutrient mineral elements in the diet of chickens on feather pulling and cannibalism. *Poultry Science*, 32: 309-313.
- Wilson, S.C. and Sharp, P.J., 1975. Changes in plasma concentrations of luteinizing hormone after injection of progesterone at various times during the ovulatory cycle of the domestic hen (*Gallus domesticus*). *Journal of Endocrinology*, 67: 59-70.
- Wilson, S.C. and Sharp, P.J., 1976. Effects of androgens, oestrogens and deoxycorticosterone acetate on plasma concentrations of luteinizing hormone in laying hens. *Journal of Endocrinology*, 69: 93-102.

- Wood-Gush, D.G.M., 1971. The behaviour of the domestic fowl. Heinemann Educational Books Ltd, London.
- Wood-Gush, D.G.M. and Gilbert, A.B., 1969. Oestrogen and the pre-laying behaviour of the domestic hen. *Animal Behaviour*, 17: 586-589.
- Wood-Gush, D.G.M. and Gilbert, A.B., 1975. The physiological basis of a behaviour pattern in the domestic hen. *Symp. Zool. Soc. Lond.*, 35: 261-276.
- Wood-Gush, D.G.M. and Rowland, C.G., 1973. Allopreening in the domestic fowl. *Revue Comportement Animal*, 7: 83-91.
- Wurdinger, I., 1982. Olfaction and home learning in juvenile geese (*Anser*- and *Branta*-species). *Biology of Behaviour*, 7: 347-351.
- Yasutomi, M. and Adachi, N., 1999. Effects of playthings on prevention of cannibalism in rearing chickens. *Japanese Poultry Science*, 372-373.

Appendix A

Definitions of behaviour categories

Ethogram

The abbreviations in parenthesis are the codes used in KEYBEHAVIOUR.

Sitting (si): sitting with the legs tucked under the body, awake or sleeping

Standing (s): standing only

Walking (w): walking or running around the pen

Pecking litter (pl): pecking at litter while standing, walking or sitting

Feeding/feeder directed (f): pecking at food in the hopper, or pecking the hopper itself

Drinking/drinker directed (dr): drinking or pecking at the drinker

Preening standing (pr): preening while standing

Preening sitting (ps): preening while sitting

Dustbathing (db): dustbathing

Scratching (sc): scratching at the litter with the feet

Aggressive pecking (ag): delivering an aggressive peck or pecks to a pen-mate

Non-aggressive pecking (na): delivering a gentle or vigorous non-aggressive peck or pecks to a pen mate

Pecking litter and *scratching* were usually combined as a measure of litter directed activity

Bird to bird pecking

Non-aggressive peck: gentle or vigorous pecks, aimed at any part of the body, often repeated, recipient may eventually withdraw.

Aggressive peck: forceful pecks, aimed at the head or back of the recipient, rarely repeated, recipient immediately withdraws (often with vocalisation).

Feather pulling: vigorous pulling of feathers of another bird (with or without subsequent feather removal).

Feather eating: ingestion of a feather (or part of a feather) regardless of source (for example the litter floor or pulled from another bird).

Appendix B

Oestradiol assay protocol and validation

Plasma oestradiol was determined using a commercial double antibody radioimmunoassay kit (Pantex ¹²⁵I Estradiol, Biogenesis Ltd, Poole, UK), which is designed for clinical use in humans. To improve accuracy, radioimmunoassay kits to determine plasma oestradiol normally incorporate an extraction step which serves to remove interfering substances (for example binding proteins and other plasma proteins which influence antigen-antibody binding) and standardise assay conditions in standard and sample tubes. Because laying hen plasma contains considerably higher levels of lipid and lipoproteins than human plasma (up to 30mg/ml), the extraction procedures designed for use with human or mammalian plasma have limited effectiveness. Therefore, a modified extraction procedure was employed in conjunction with the commercial kit, after various comparisons had been carried out to identify the most effective solvents and the best and solvent: plasma ratio.

Extraction procedure

The extraction used was similar to the solvent extraction described by Webb et al., (1985). Plasma samples (0.3ml) were equilibrated with recovery label (10ul tritiated oestradiol in ethanol, approximately 1000 cpm) for 30 minutes in borosilicate glass extraction tubes. 5ml hexane/diethyl ether (HPLC grade, Rathburn Chemicals) (4:1) was added to each sample, tubes were capped with teflon lined caps and vigorously mixed on a multivortexer (SMI) for 30 minutes. After mixing, tubes were left to stand until a clean aqueous/solvent interface was visible. The aqueous phase was frozen in a dry ice-methanol bath and the solvent phase decanted into clean glass tubes and evaporated to dryness in a Buchler vortex evaporator. Samples were then reconstituted with 800ul of assay buffer (supplied with kit, pH 7.4) and vortexed at 40°C for 20 minutes to solubilize dried oestradiol. A proportion of the reconstituted (200ul) sample was used to estimate recovery.

Mean extraction efficiency, as determined by recovery of tritiated oestradiol calculated in each tube was $73 \pm 0.06\%$ (n=200). Individual recoveries were taken into account in the final calculation of oestradiol concentration for every sample.

Summary of assay protocol

The double antibody radioimmunoassay procedure described in the kit protocol was followed. Standards (512, 256, 128, 64, 32, 16, 8, 4 pg/tube) were made up by halving dilutions of a standard solution made up with stock standard (51,200pg/ml) supplied with the kit. Replicate standards and extracted samples (500ul) were assayed in glass tubes (12x25mm) by addition of tracer (iodinated oestradiol) and first antiserum and incubation at 37°C for 1 hour. Addition of a second antiserum was followed by a 20 minute incubation at room temperature. After centrifugation at 3500rpm for 10 minutes, the supernatant was removed from each tube by aspiration and the ¹²⁵I content of the pellets determined using a gamma counter (1277 Gammamaster, Wallac). Appropriate calculations to determine oestradiol concentration in samples were carried out on a spreadsheet.

Assay Validation

Accuracy of oestradiol measurement in avian plasma was evaluated by adding 1536, 1024, 512 and 256 pg oestradiol to separate 1ml aliquots of pooled plasma (from 10 pullets aged 10-16 weeks). The spiked samples were extracted and assayed in duplicate as described above. The pooled plasma was also extracted and assayed, and was found to contain 196pg/ml, which was deducted from the spiked sample concentrations. The results are shown in Table AC.1

Oestradiol added to 1ml pooled plasma (pg)	0	1536	1024	512	256
Mean oestradiol measured (pg/ml)	196	1557	930	543	223
% accuracy	—	101	91	106	87

Table AC.1 Measurements of oestradiol in spiked pooled plasma samples.

These results show a mean accuracy of 96%. Pooled plasma samples (as above) were included in all assays as a measure of quality control. Inter-assay coefficient of variation of 10.7% (n=8) was observed. The intra-assay coefficient of variation was 5.2%.

Appendix C

Age related changes in targeting of non-aggressive pecks

The figures below show age-related changes (from 5 to 26 weeks) in the targeting of non-aggressive (feather) pecks at the back, tail, wings, neck and beak in growing ISA Brown pullets (data from Chapter 3).

