# FINAL REPORT

## EGG INDUSTRY RESEARCH AND DEVELOPMENT COUNCIL

**PROJECT UF 4E**

# EFFECTS OF FOOT LESIONS AND FEATHER LOSS ON THE WELFARE OF CAGED LAYERS USING ANATOMICAL AND BEHAVIOURAL APPROACHES.

by

**DR CHRISTINE A LUNAM**

DEPARTMENT OF ANATOMY AND HISTOLOGY
SCHOOL OF MEDICINE
FLINDERS UNIVERSITY
BEDFORD PARK SA 5042
TELEPHONE: 61-8-82044704

and

**DR PHILIP C GLATZ**

SOUTH AUSTRALIAN RESEARCH AND DEVELOPMENT INSTITUTE
ROSEWORTHY SA 5371

APRIL 1997

## TABLE OF CONTENTS

# EXECUTIVE SUMMARY

The effects of poor feather cover and foot lesions on the well-being of caged hens were examined. Well-being was assessed by behavioural parameters and histopathology of the effected areas. Three experiments were conducted. Two experiments examined the effect of poor feather cover on the well-being of two different strains of laying hens at 70 weeks of age. The first experiment examined an Australian strain whilst the second experiment assessed a European Brown strain. The third experiment assessed the effects of foot lesions on the well-being of an Australian strain.

## Poor feather cover-Australian strain

Behaviour and histopathology of hens (70 weeks of age) with poor feather cover were compared with hens with good feather cover.

- No significant differences in sitting, preening, hen pecking, cage pecking, eating and drinking behaviour or incidence of head scratching, dust bathing, feather ruffling could be detected.
- There was a trend for the hens with poor feather cover to engage in less preening.
- No differences were observed in the thickness and number of dividing cells in well-feathered skin compared to skin of the same region that was poorly-feathered. This suggests that removal of the feathers does not cause excessive abrasion of the underlying skin.
- The lack of increased inflammation in the poorly feathered skin compared to that in well feathered skin suggests poorly feathered regions are not subject to long term infection.
- Silver staining demonstrated numerous nerves in all skin examined. Some of these nerves ended freely near the surface of the skin. Immunohistochemical staining revealed some of the nerves contained peptides such as substance P. The location of some nerves containing substance P in the skin indicates that these nerves are capable of transmitting pain. The anatomical findings suggest that rapid removal of feathers by hen pecking is likely to induce acute pain at the time of feather removal. However, the lack of other histopathology changes in the skin suggest that feather abrasion does not induce long term chronic pain.

*These observations indicate that hens with poor feather cover show no obvious anatomical or behavioural signs indicating they are feeling persistent pain as a result of their body condition.*

## Poor feather cover-European Brown strain

Behaviour and histopathology of hens (70 weeks of age) with poor feather cover was compared with hens with good feather cover. Behavioural and anatomical parameters measured were that conducted for the Australian strain.

- No major differences in behaviour could be detected, except hens with good feather cover engaged in more bouts of pecking at the cage.
- There was a trend for hens with good feather cover to peck more at other birds.

- The anatomy of the skin was similar to that of the Australian strain, with a similar distribution of nerves. The lack of signs of response to injury of the skin after feather removal, that is no visible signs of either thickening of the skin nor increased inflammation, suggests that the skin is neither excessively abraded nor is more prone to infection following feather removal.

*These observations indicate that hens with poor feather cover show no obvious anatomical or behavioural signs indicating they are feeling persistent pain as a result of their body condition*

## Comparison of Australian and European Brown strain

- No differences were observed in the structure or nerves of the skin between the strains. Similarly, the skin of both strains showed no response to feather removal. This suggests that both the Australian and European strains are unlikely to be subject to chronic pain as a result of feather loss.
- The European Brown strain was more aggressive to its cage mates with significantly more bouts of hen pecking compared to the Australian strain. In addition, the European strain spent more time sitting and engaged in more head shaking compared to the Australian strain.

## Effects of foot lesions on the well-being of an Australian strain of caged layers

To assess the effect of foot lesions on the well-being of commercial caged laying hens (70 weeks of age), we compared the behaviour of hens and histopathology of the toes with foot lesions to a control group having no foot lesions.

- Foot lesions were found in 20 hens out of a total group of 2000.
- Hens with foot lesions had significantly more drinking bouts of less duration per bout. A possible explanation is that the foot lesions become sore when additional pressure was placed on them as the hens reached to drink from the water nipple.
- Hens with foot lesions spent significantly more time feather ruffling than hens without foot lesions. The increased time spent feather ruffling may be a displacement activity to alleviate distress caused by foot lesions.
- No significant differences in bouts of sitting, preening, hen pecking, cage pecking and eating or incidence of head scratching or dust bathing was observed between the treatments.
- Histology of the toes revealed an often marked inflammatory response associated with the site of the lesion. This indicates that the site of the lesion is more prone to infection compared to regions of the foot without lesions.
- Immunohistochemistry revealed individual nerve fibres of the type capable of transmitting pain in all toes examined, that is they contained the peptide substance P.

*Inflammation and the presence of nerves with characteristics features of fibres capable of transmitting pain, suggest that foot lesions are likely to be persistently painful. This proposal is supported by the behavioural changes in drinking and preening behaviour.*

*In summary our observations indicate that the well-being of the caged hens is likely to be compromised by the presence of foot lesions.*

The first section of this report outlines the factors that contribute to feather cover and foot lesions, as well as addresses the economic and welfare implications of these conditions. Sections I to IV describe the experiments undertaken in this study. Sections I, II, and III describe the anatomical and behavioural effects of poor feather cover in two strains of caged layers. Section IV examines the behavioural and anatomical effects of foot lesions on caged layers and discuss what these effects mean in terms of well-being.

# BACKGROUND

## Feather cover

It is well known that feather cover of laying hens declines as the bird ages (Tauson 1986). By the end of lay some hens are almost naked. When hens have poor feather cover during the cooler months food intake increases by 6% to compensate for the increased heat loss from hens (Lee *et al* 1983). In Australia half of the total hen population are older hens and they could be consuming up to 7 g/b/d more food than necessary during the cooler months amounting to extra food costs for the egg farmers of approximately $1.5m. Thus maintaining the plumage of hens has economic importance in addition to aesthetic benefits. Preliminary work undertaken in Australia by Woolford *et al* (1990) indicated that while age and strain were key factors influencing feather cover it could only explain 28% of the variation. Clearly there are a number of other factors influencing the degree of feathering that could be manipulated at the farm level to improve feather cover.

## Feather pecking

Feather pecking has always been a serious problem of poultry. It consists of pecking directed at feathers of other birds sometimes involving plucking out and eating these feathers. It may result in severe damage of the birds with bare patches and wounds to the skin in more serious pecking attacks eventually leading to death of the bird. Frequency of pecking is high in cages (Koelke *et al* 1987) and while cannibalism is generally controlled, feather cover in older birds is quite poor, creating a poor image of the caged hen to the public. Hens housed in cages also lose feathers from back scratching (Alvey and Tucker 1993). Gentle and Hunter (1990) reported feather removal is likely to be painful. This is based on observed changes in blood pressure, heart rate and brain wave patterns following feather removal. However, Gentle and Hunter (1990) also reported that birds being feather pecked show little or no behavioural responses that indicate removal of feathers is painful.

We will examine the nerves that innervate the body area in question to determine whether they correspond to nerves that are likely to transmit pain. Thus, the morphological characterisation of the nerves provides a powerful basis for understanding the behavioural response of the bird. Thus there is an urgent need to identify the types of nerves that innervate the skin of the bird to clarify the true welfare of birds with minor injuries.

## Ground pecking

Feather pecking is a form of redirected ground pecking (Blockhuis 1986,1989). Ground pecking apart from food gathering is a form of exploratory behaviour serving to gather visual, tactile and gustatory information. Increasing the incentive value of the ground by using straw or grain for floor reared pullets significantly reduced feather damage in the laying period (Blockhuis and Van Der Haar 1989). Use of grain for instance directs foraging-related behaviours like scratching and pecking to the ground ultimately resulting in less feather pecking in the adult phase. Rearing on litter causes hens to feather peck less than hens reared on wire floors (Blokhuis and van Der Haar 1989).

## Stereotype behaviour

Feather pecking is considered to be stereotypic behaviour. Some birds develop very high frequencies while other show very low frequencies (Kostal *et al* 1992). This may account for the wide variation in feather cover noted in the field. Genetic variation also affects feather pecking and feather loss (Craig 1992).

## Relation to dust bathing

Vestegaard *et al* (1993) reported that feather pecking was most likely to occur in the Red Jungle fowl when birds were dust bathing or preparing to do so. Severe feather pecks received during dust bathing were correlated with the amount of feather damage on the recipient. Birds that did the most feather pecking were the ones that did the least dust bathing and were also the most fearful. Allopreening pecks (gentle pecks) can be easily distinguished from the severe feather pecks and both types of feather pecking were related to social status of the bird.

## Use of stock wound

For many years poultry farmers have been using antiseptic coloured sprays to treat pullets and hens suffering from injuries caused by cannibalism or scratches and abrasions from wire cages. There is considerable anecdotal evidence that these stock wound sprays prevent further aggressive feather pecks from cage mates and feathers begin to grow back in the sprayed area. We believe more extensive use could be made of these sprays on poorly feathered birds especially late in lay and could prevent further feather pecking and improve feather cover.

In combination with good beak trimming, appropriate rearing techniques and use of abrasive strips we believe there is considerable scope to improve the feather cover of older hens, reduce food costs, improve efficiency and the appearance of the laying hen. Results of this work could have immediate application to the Industry.

## Beak trimming

Beak trimming not only reduces the amount but also the effectiveness of feather pecking resulting in a lower degree of feather deterioration (Hughes and Michie 1982). Flocks inadequately trimmed generally have higher mortality from peck-outs and cannibalism because hens are able to inflict more damage with their beaks. Recent work by Glatz (1993) showed that age of trimming influenced amount of feather pecking.

Studies examining the effect of light intensity on feather pecking and feather cover are scant. Tauson(1986) reports light intensity including temperature influence feather cover. It is well known in industry that reducing light intensity reduces the need to beak trim poultry as is the case in many European countries. Visitors from Europe have questioned the need to beak trim commercial poultry in Australia. However light intensity is very low in their shedding, and very high in our sheds. In recent years the Europeans have found that their supposedly docile strains when housed outdoors in alternative systems develop severe cannibalism problems necessitating the need to beak trim. Quite clearly light intensity has a huge impact on feather pecking but a quantitative relationship between light intensity, feather pecking and feather cover has not been defined in the literature.

## Controlling growth of claws

Bird's claws grow continuously and cage floors do not allow for the on-going wearing down that occurs in floor housed birds. With long claws birds can injure themselves and cage mates and run the risk of getting trapped in the cage structure. Long twisted claws are frequently quoted as a negative aspect of caging. Tauson (1986) reported a low-cost, non-invasive, method by which the claws of caged layers could be kept short and blunt through the laying year. He recommended sticking an 8 mm strip of abrasive tape on the egg guard. Bird's claws scraped against this tape while they were feeding. This technique offers positive welfare advantages for bird and removes a criticism of cages. Many cages in Australia do not have egg guards and abrasive strips will need to be fitted to the feed trough.

## Foot disorders in layer hens

Most lesions can be separated into two main classes. Hyperkeratosis (thickening of the skin) is often present both on the toe and foot pads of birds kept in cages (Tauson 1980), whereas severe inflammation and swelling tends to occur on the foot pad. Causes of foot lesions have been attributed to dietary deficiency (Burger *et al* 1984), stocking density and cage floor design (Tauson 1980), perches (Appleby *et al* 1992) and housing system (Tauson and Jansson 1988).

## Behaviour as a measure of welfare

Behaviour has been used as a non-invasive technique to give an indication of the welfare of the bird. There is increasing acceptance that the animal's behaviour is the best "window" of its feelings and is a reasonable method to identify suffering in animals. However it has been shown that sometimes behaviour indicative of pain needs careful interpretation when associated with beak trimming in layers (Duncan *et al* 1986, Gentle *et al* 1990, Glatz 1990). Initial responses of chicks to trimming for instance, are not indicative of a pain response.

Our approach is to assess the behavioural activities of hens with various body conditions. These behavioural profiles will indicate the responses hens make in their environment when faced with injury. Anatomical studies will provide an indication of the underlying cause of the response, such as the presence or absence of inflammation, excessive thickening of the skin and presence of nerves associated with pain conduction. By comparing anatomical findings with behaviour we can determine the likelihood of the type and degree of discomfort experienced by the hens.

## What is known regarding the skin covering feathered regions and feet of layers.

The hen is covered by skin and specialised derivatives of skin to form the claws, scales and feathers. In contrast to mammals and many other vertebrates the skin of fowls is very loose and thin. Birds have no sweat glands to cool and protect the skin and keep it moist. The feathers of the fowl are kept moist by a single gland, the uropygial gland that aids in preening and water proofing the feathers.

A major function of the skin is to act as first line of defence against mechanical damage and invasion by pathogens. In addition the skin is the largest organ of the body that contains numerous receptors for touch, pressure and pain. Each of these receptors is innervated by its own specific type of nerves that are distinguishable from one another by size, electrical activity, presence or absence of a myelin sheath and by the chemicals that they contain.

Pain fibres belong to the C and A-delta classes of nerves, these are small diameter unmyelinated and small diameter myelinated nerves respectively. In mammals as well as in the beak of chicks (Lunam and Glatz 1993, 1995) they appear as free nerve endings near the epidermis, the outer layer of the skin. These free nerve endings (nociceptors) transmit pain from the skin to the spinal cord and brain. These nerves contain specific combinations of chemicals that include the neuropeptides substance P and calcitonin-gene related peptide (Lunam and Glatz 1993); other peptides such as galanin and neurokinen A have also been found in similar nerves in mammals.

## Immunohistochemical identification of putative pain fibres

As "pain fibres" are very small they are often missed by conventional histological stains. Immunohistochemistry is a specialised technique that can be used at the light (Lunam 1993) and electron microscope levels. As it is highly specific it allows identification of the different types of nerves by tagging the chemicals they contain. Thus free nerve endings that contain substance P (fibres that transmit pain) can be distinguished from other types of nerves.

# OBJECTIVES OF RESEARCH

1. To examine the histopathology of skin abrasion from feather loss (via either pecking or cage abrasion) and foot lesion.
2. To determine the type and extent of nerves, specifically to identify nerves containing substance P, in the regions of the feathered skin and foot using specialised light microscope techniques, immunohistochemistry and silver staining.
3. To determine the effect of body injury on behaviour of layers using a variety of standard criteria.
4. To provide the Egg Industry with an index of welfare/putative pain of the caged hen with either feather abrasion or foot lesions.

# INTRODUCTION

The keeping of hens in cages has attracted severe criticism from both welfare groups and the general public. In particular, feather loss and foot lesions have been criticised as they may cause pain in caged hens. Feather loss of caged layers has both economic and welfare implications for the Australian Egg Industry. To maintain body temperature, hens having poor feather cover may during the cooler months increase their food intake by 6% more than hens with good feather cover. Subsequently, in Australia, hens could be consuming up to 7 g/b/d more food than necessary during the cooler months. This increased food consumption is estimated to cost farmers an additional $1.5m annually. Therefore maintaining the plumage of hens has economic importance in addition to aesthetic and welfare benefits.

Direct comparison of behaviour with anatomy was employed to provide meaningful data on the welfare of hens with specific body injury. In this study the histopathology and presence of nerves in the skin and feet of 70-week-old caged hens with either poor feather condition or foot lesions were compared to hens without body injury. The presence and distribution of nerves was determined by silver impregnation and the immunohistochemistry technique was employed to identify potential pain fibres by visualising the chemical content of the nerves.

# SECTION I: EFFECTS OF POOR FEATHER COVER ON BEHAVIOUR AND ANATOMY IN AN AUSTRALIAN STRAIN OF CAGED HEN.

## Introduction

The keeping of hens in cages and the use of various husbandry practices has attracted severe criticism from welfare groups and the general public. The main criticism of the caged bird is that wire cages cause discomfort and injury as the feathers are rubbed off and the skin abraded by the wire. Skin normally covered with feathers is very thin. Consequently an important function of feathers is to protect the underlying skin from abrasion and invasion by parasites and pathogens (Lucas and Stettenheim 1972). Protection of the skin is essential to the well-being of the bird as the skin contains blood vessels, sensory receptors and nerves. Gentle and Hunter (1990) reported feather removal is likely to be painful, as they observed changes in blood pressure, heart rate and brain wave patterns following feather removal. However, long term effects on behaviour of hens with poor feather cover have not been examined.

The aim of this study was to assess the longer-term effects of feather abrasion on the welfare of caged hens. In this study, we correlated the histopathology of the skin of the neck region to the behaviours of the hens. Immunohistochemsitry was conducted to determine the distribution of putative pain fibres, that is nerves labelling for substance P and/or calcitonin-gene related peptide. Behavioural studies were done on the basis of overall body scores. We examined hens with different feather conditions; these ranged from complete feather cover to those with almost complete feather loss. No discrimination was made between the removal of feathers from either cage abrasion or by pecking from other hens.

## Materials and methods

### *Birds and management*

Forty hens (70 weeks of age) were selected from a caged flock of 2000 commercial laying hens and allocated in pairs to single tier laying cages (45 by 45 by 40 cm) and maintained on a layer ration. The hens were segregated into 2 groups (n=20 per group) on the basis of overall body feather cover for behavioural studies. Histological data are reported here for the neck region.

Feather cover was scored in the range of 0 for almost total loss of feathers to 5 for full feather cover. A grade of 0-3 was considered as poor feather cover and skin condition, group 1; whereas a grade of 4 or 5 was considered good feather cover and skin condition, group 2.

*Video recording of behaviour; viewing video tapes; analyses*

A video recording was made for each pair of hens in each treatment post-lay from 1300h-1600h with food and water available *ad libitum.* Data on behaviour were obtained from watching video records and manually keying observations into a hand held micro-computer. The activities recorded were time and bouts of pecking at food, drinking, preening, sitting and number of pecks made at the cage and other birds. Two separate bouts of behaviour were recorded if they were separated by a pause of at least 5 sec duration. Incidence of dust bathing, feather ruffling and head scratches were also recorded. SAS linear modelling procedures were used to analyse the effect of feather cover and skin condition on behaviour of hens.

*Histological assessment*

Immediately after killing by cervical dislocation samples of skin were taken from either the lower or mid neck of 10 hens from each of groups 1 and 2. The tissue was fixed by immersion in Zamboni's fixative (Stefanini *et al* 1967) for several days at 4°C. A small piece of skin was processed by routine wax-embedding and 5μm-thick transverse sections stained with either haematoxylin and eosin, or Verhoeff and van Gieson for visualisation of tissues types and any inflammatory response. A second piece of skin was processed for the identification of nerve fibres using a triple silver impregnation stain (Gilbert 1965) on frozen transverse-sections of 40 μm-thickness.

*Immunohistochemistry*

A piece of skin from each hen was processed for immunohistochemical identification of nerve fibres labelling for substance P (SP). After clearing in dimethyl sulphoxide, the tissue was washed in 0.01M phosphate buffered saline (NaCl: 8.5g/l), and 10 micrometer - thick frozen sagittal sections collected and labelled as described previously (Lunam 1989, 1993). Primary antibodies were rat anti-SP (1:300) and rabbit anti-calcitonin gene-related peptide (CGRP, 1:2000). Secondary antibodies were goat anti-rat TRITC- conjugated, Cappel at 1:80 and sheep anti-rabbit FITC-conjugated, Silenus Australia at 1:160. Cross reactivity and pre-absorption test were conducted to check that no non specific binding of the antibodies had occurred. Single labelling of substance P immunoreactivity was also conducted. In this case the primary antibody was rabbit anti-substance P, 1:2000 (Silenus) and the secondary antibody was sheep anti-rabbit FITC-conjugated, Silenus Australia at 1:160.

## Results

*Behaviour*

Hens with poor feather cover showed no significant difference in sitting bouts, preening bouts, hen peck bouts, cage peck bouts, eating bouts and drinking bouts compared to hens with good feather cover. Variables which approached significance between the treatments (Table 1) were preening bouts (P=0.09) and drinking bouts (P=0.12).

**Table 1**. Effects of poor feather cover (PFC) and good feather cover (GFC) on number of bouts per h of sitting (SB), preening (PB), hen pecking (HPB), cage pecking (CPB), eating (EB) and drinking (DB). P = probability value in analysis of variance.

| Treatment | SB | PB | HPB | CPB | EB | DB |
|---|---|---|---|---|---|---|
| PFC | 4.4 | 20.3 | 6.7 | 2.1 | 22.4 | 12.6 |
| GFC | 3.6 | 25.8 | 6.1 | 2.6 | 23.6 | 9.2 |
| P | 0.39 | 0.09 | 0.65 | 0.55 | 0.64 | 0.12 |

Hens with poor feather cover showed no significant differences in time spent sitting, preening, eating, drinking, incidence of feather ruffling, head scratching or dust bathing. Time spent preening (P=0.09) and incidence of head scratching (P=0.13) approached significance between the treatments (Table 2).

**Table 2.** Effects of poor feather cover (PFC) and good feather cover (GFC) on time (sec) spent sitting (ST), preening (PT), eating (ET), drinking (DT) and incidence of feather ruffling (FR), head scratching (HS) and dust bathing (DB) averaged over 1h. P = probability in analyses of variance.

| Treatment | ST | PT | ET | DT | FR | HS | DB |
|---|---|---|---|---|---|---|---|
| PFC | 705 | 546 | 733 | 266 | 0.4 | 6.6 | 1.0 |
| GFC | 807 | 654 | 749 | 247 | 0.4 | 5.3 | 0.4 |
| P | 0.53 | 0.10 | 0.85 | 0.70 | 0.94 | 0.13 | 0.29 |

## Histopathology

No differences were observed in the histology of skin taken from either the mid or lower regions of the ventral surface of the neck from either group. Other than the difference in numbers of feather follicles and the associated muscle and elastic fibres, the morphology of skin in which the feathers had been almost totally removed, could not be discerned from skin with total feather cover using either haematoxylin and eosin, or Verhoeff and van Gieson stains.

The skin of the neck was thin, the keratinised epidermis consisting of three to four layers of cells. The epidermis was intact in all sections and showed no evidence of excessive abrasion. Similar numbers of mitotic figures were present in the stratum germinativum of the epidermis of all hens examined. A thick dense band of collagen, 15 to 40 μm in width, was observed running immediately beneath and parallel to the epidermis. Beneath the band of collagen the dermis consisted of dense irregular collagen bundles supporting numerous blood capillaries and venules. Macrophages and small aggregations of lymphocytes were observed in all tissues examined. Eosinophils were numerous in the dermis of two hens with poor feather cover. Associated with the feather follicles were smooth muscle bundles that inserted via elastic tendons into both the adipose and dense connective tissue, and the follicular sheaths .

Silver impregnation revealed numerous nerve bundles in the dermis and subdermal connective tissue. The nerves were frequently associated with blood vessels and feather follicles. No differences were observed in either the distribution or numbers of nerve bundles in skin having poor feather cover compared to skin with good feather cover.

Sparse nerve fibres labelling for SP were present in the dermis and free nerve endings were visible at the dermal epidermal junction. Nerve fibres labelling for calcitonin-gene related peptide were not detected. SP-IR fibres were situated along the walls of arteries and veins. SP-immunoreactive nerve were not associated with the muscle associated with the feather follicles.

## Discussion

Hens with poor feather cover demonstrated no changes in behaviour compared to hens with full feather cover. These results indicate that considerable feather loss does not compromise the long-term well-being of caged hens. Although no statistically significant differences in behaviour were observed between the two groups of hens, hens with poor feather cover showed a reduction in preening which approached significance, $p=0.09$. Indeed, hens with less covering feathers would be expected to reduce the time spent grooming.

Our lack of behavioural differences between the two groups of hens is in agreement with the histological findings. The thickness of the epidermis as well as the number of mitotic figures were similar in well-feathered and poor-feathered skin. These findings suggest that the featherless skin is not being excessively abraded by the wire. If this were the case, the epidermal cells would be expected to undergo an increased rate of mitosis, causing an increase in thickness of the skin, in an attempt to replace excessive sloughing of the damaged epidermal cells. In addition, with the exception of a few sections from skin with very poor feather cover, the number of macrophages and eosinophils were similar between the two groups. The lack of inflammatory response supports our other histological findings that feather abrasion does not induce injury to the skin.

Although it is well known that sympathetic nerves supply the feather muscles, the *mm. pennarum,* little is known regarding the sensory innervation of the skin. Silver staining revealed an abundance of nerves fibres in the dermis and subdermal tissue of the neck skin of all hens examined. No nerve fibres however penetrated beyond the dermis into the epidermis. Immunohistochemistry revealed that some nerve fibres ending at the dermal epidermal junction contain substance P indicating they are nociceptors that transmit painful stimuli. It was surprising that these fibres did not label for calcitonin gene-related peptide as in mammals many putative pain fibres label for both peptides. The possibility cannot be excluded that these nerves do contain calcitonin gene-related peptide or that this peptide was not recognised by the antibody used. However, previous studies in other tissues (Lunam 1995) have determined that this antibody does recognise chicken calcitonin gene-related peptide.

In summary, our histological and behavioural data to date, strongly indicate that feather-abrasion of the neck region does not compromise the well-being of caged hens.

# SECTION II: EFFECTS OF POOR FEATHER COVER ON BEHAVIOUR AND ANATOMY IN A EUROPEAN BROWN STRAIN OF CAGED HEN.

## Introduction

To assess the effects of strain differences on feather cover and well-being of caged hens, we compared behavioural and anatomical parameters previously described (SECTION I) for an Australian layer strain to that for a brown European strain.

## Materials and Methods

*Hens*

Forty hens at 70 weeks of age were selected from a commercial caged flock and allocated in pairs to single tier laying cages (45 by 45 by 40 cm) and maintained on a layer ration. The hens were segregated into two treatments on the basis of overall body feather score using a system similar to that described by Tauson (1984). Feather cover was scored in the range of 0 for almost total loss of feathers to 5 for full feather cover.

*Video recording of behaviour, viewing video tapes, analyses*

A video recording was made for each pair of hens in each treatment post lay from 1300h-1600h with food and water available *ad libitum*. Data on behaviour were obtained from video records by manually keying observations into a micro-computer. Activities recorded were time and bouts of pecking at food, drinking, preening, sitting and number of pecks made at the cage and other birds. Two separate bouts of behaviour were recorded if they were separated by a pause of at least 5 sec duration. Incidence of dust bathing, feather ruffling and head scratches were also recorded. SAS linear modelling procedures were used to analyse the effect of poor feather cover on behaviour of hens. Additional measurements made were head shakes, tail wags, bill wipes, wing and leg stretches, pecks to cage mate, pecks to birds in other cages and pecks received.

*Histological assessment*

Immediately after killing by cervical dislocation samples of skin were taken from either the lower or mid neck of 10 hens from each of groups 1 and 2. The tissue was fixed by immersion in Zamboni's fixative (Stefanini *et al,* 1967) for several days at 4°C. A small piece of skin was processed by routine wax-embedding and 5μm-thick transverse sections stained with either haematoxylin and eosin, or Verhoeff and van Gieson for visualisation of tissues types and any inflammatory response. A second piece of skin was processed for the identification of nerve fibres using a triple silver impregnation stain (Gilbert, 1965) on frozen transverse-sections of 40 μm-thickness.

## Results

Hens with poor feather cover showed no significant difference in sitting bouts, preening bouts, hen peck bouts and eating bouts compared to hens with good feather cover. The only variable which was significantly different between the treatments (Table 3) was cage peck bouts (P=0.01). The hens with good feather cover engaged in more bouts of cage pecking than hens with poor feather cover.

**Table 3**. Effects of poor feather cover (PFC) and good feather cover (GFC) on the number of bouts per h of sitting (SB), preening (PB), hen pecking (HPB), cage pecking (CPB), eating (EB) and drinking (DB). P= probability value in analysis of variance. Means within columns followed by a different letter are significantly different (P=0.05).

| Treat | SB | PB | HPB | CPB | EB | DB |
|---|---|---|---|---|---|---|
| PFC | 3.5 | 19.5 | 10.7 | 2.5a | 21.6 | 9.9 |
| GFC | 4.8 | 22.5 | 17.1 | 11.7b | 19.0 | 12.4 |
| P | 0.38 | 0.48 | 0.38 | 0.01 | 0.45 | 0.44 |

Similarly hens with poor feather cover showed no significant differences in time spent sitting, preening, eating, drinking, head scratching and dust bathing (Table 4).

**Table 4.** Effects of poor feather cover (PFC) and good feather cover (GFC) on time (sec) spent sitting (ST), preening (PT), eating (ET), drinking (DT) and incidence of feather ruffling (FR), head scratching (HS) and dust bathing (DB) averaged over 1h. P = probability in analyses of variance.

| Treat | ST | PT | ET | DT | FR | HS | DB |
|---|---|---|---|---|---|---|---|
| PFC | 369 | 470 | 740 | 185 | 0.43 | 3.7 | 1.3 |
| GFC | 540 | 431 | 696 | 131 | 0.69 | 2.4 | 0.7 |
| P | 0.31 | 0.70 | 0.79 | 0.22 | 0.58 | 0.11 | 0.43 |

No differences were found in the histological appearance of the skin between full feathered and feather abraded birds. The thickness of the epidermis as well as the number of mitotic figures were similar in well-feathered and poor-feathered skin. These findings suggest that the featherless skin is not being excessively abraded by the wire. If this were the case, the epidermal cells would be expected to undergo an increased rate of mitosis, causing an increase in thickness of the skin, in an attempt to replace excessive sloughing of the damaged epidermal cells. In addition the number of macrophages and eosinophils were similar between the two groups. The lack of inflammatory response supports our other histological findings that feather abrasion does not induce injury to the skin.

## Discussion

These studies examined if hens from a European brown strain with poor feather cover showed any major changes in behaviour or histology which indicate the hens were in pain. No major differences in behaviour could be detected, except hens with good feather cover engaged in more bouts of pecking at the cage. Although not significant, there was a trend for hens with good feather cover to peck more at other birds whereas birds with poor feather cover engaged in fewer bouts of pecking at other birds. A possible explanation for this difference in pecking behaviour may be that birds with poor feather cover are more vulnerable to both abrasion by the cage and pecking by full feathered birds and are therefore less aggressive. From an Industry point of view it could be good husbandry practice towards end of lay in middle of winter to segregate birds with poor and good feather cover. This may result in improvement of feather condition and could reduce food intake of hens as they regain feather condition. Reduction in pecking by hens with good feather cover could be reduced by placement of enrichment devices in the cage.

Silver impregnation revealed nerve fibres that ended freely in the dermis, suggesting that these are likely to have a nociceptive function. For nociceptive function, pain conducting fibres need to be stimulated either by direct injury or by tissue mediators associated with infection. The lack of any noticeable inflammatory response in the skin with poor feather cover compared to similar regions of well feathered birds suggests that pain associated with infection is unlikely.

# SECTION III: COMPARISON OF WELL-BEING OF TWO STRAINS OF CAGED HENS WITH POOR FEATHER COVER: EUROPEAN BROWN VERSUS AUSTRALIAN.

## Materials and Methods

*Pooling of behavioural data*

Separate experiments were conducted with an Australian strain and the European brown strain (see sections I and II). These experiments were conducted under the same housing and temperature conditions but at a different time. Data was pooled to compare the behaviour of the European strain with the Australian strain.

*Histopathology*

Histological sections taken from both strains with both good and poor feather cover were compared for histopathology using silver impregnation of nerves viewed with Nomarski optics to identify the distribution of nerves and their relationship to structures within the skin. Haematoxylin and eosin stains were used to assess the presence or absence of inflammation (Sections I and II).

## Results

**Table 5.** Effects of poor feather cover (PFC) and good feather cover (GFC) and strain on the number of bouts per h of sitting (SB), preening (PB), hen pecking (HPB), cage pecking (CPB), eating (EB) and drinking (DB). P= probability value in analysis of variance. Means within columns followed by a different letter are significantly different (P=0.05).

| TREAT | SB | PB | HPB | CPB | EB | DB |
|---|---|---|---|---|---|---|
| PFC | 3.7 | 20.1 | 11.7 | 2.3a | 22.2 | 11.7 |
| GFC | 4.5 | 23.6 | 8.6 | 11.6b | 20.9 | 10.6 |
| P | 0.40 | 0.29 | 0.39 | 0.001 | 0.60 | 0.65 |
| STRAIN | | | | | | |
| Australian | 4.0 | 22.7 | 6.4a | 6.8 | 22.9 | 11.1 |
| European | 4.1 | 21.0 | 13.9b | 7.1 | 20.3 | 11.2 |
| P | 0.91 | 0.61 | 0.04 | 0.92 | 0.29 | 0.94 |

**Table 6** . Effects of poor feather cover (PFC) and good feather cover (GFC) on time (sec) spent sitting (ST), preening (PT), eating (ET), drinking (DT) and incidence of feather ruffling (FR), head scratching (HS) and dust bathing (DB) averaged over 1h. P= probability in analyses of variance.

| Treat | ST | PT | ET | DT | FR | HS | DB |
|---|---|---|---|---|---|---|---|
| PFC | 482 | 515 | 738 | 228 | 0.42 | 5.3a | 1.2 |
| GFC | 730 | 530 | 720 | 161 | 0.53 | 3.7b | 0.6 |
| P | 0.08 | 0.84 | 0.84 | 0.08 | 0.69 | 0.008 | 0.20 |
| STRAIN | | | | | | | |
| Australian | 758a | 594 | 740 | 232 | 0.39 | 6.0a | 1.0 |
| European | 454b | 451 | 718 | 158 | 0.56 | 3.1b | 0.8 |
| P | 0.04 | 0.08 | 0.81 | 0.06 | 0.50 | 0.001 | 0.60 |

Analyses of pooled data confirmed that hens with poor feather cover showed no significant difference in sitting bouts, preening bouts, hen peck bouts, eating and drinking bouts compared to hens with good feather cover. The only variable which was significantly different between the treatments (Table 5) was cage peck bouts (P=0.001). The hens with good feather cover engaged in more bouts of cage pecking than hens with poor feather cover.

Similarly hens with poor feather cover showed no significant differences in time spent sitting, preening, eating, drinking, feather ruffling and dust bathing compared to hens with poor feather cover (Table 6). There was however a significant increase in head scratches for hens with the poor feather cover.

Strain differences in behaviour were for the European compared to the Australian strain, an increase in hen peck bouts, a decrease in sitting time and decreased head shakes

## Discussion

Comparison of the strains revealed one significant finding of practical importance to the Egg Industry. The European strain engaged in significantly more bouts of feather pecking which supports the anecdotal evidence from the Egg Industry that in general imported strains are more aggressive than local strains. In particular it is important that the overseas strains are carefully monitored for vice behaviour and preventative husbandry strategies are put in place to avoid cannibalism. In Europe these hens were housed under low light intensity and the need for beak trimming and declawing was not necessary. Under Australian conditions there is definitely a need to beak trim and in most cases to declaw the middle toe of hens to prevent injuries to the back and abdomen.

It is clear that hens with good feather cover peck more at the cage than those hens with poor feather cover. This may only indicate that hens with good feather cover relieve their boredom by engaging in more stereotypic cage pecking behaviour compared to hens with poor feather cover. There is evidence that the Australian strain were more fearful as they had significantly higher level of head shaking and resting time.

Histopathology revealed no anatomical differences in the either structure of the skin or the response to feather loss between the European and Australian strains. Nerve fibres were present in the dermis of the skin of all tissue examined. Inflammatory responses were observed with equal frequency in skin with both full and poor feather cover. The presence of free nerve endings labelling for substance P, the characteristic "landmarks of pain transmission fibres" in the region of the feather follicle does suggest that the action of removal of the feathers by hen pecking is painful. In sum however, the anatomical evidence and behavioural findings suggest that feather abrasion (via cage or pecking by other birds) whether in either European or Australian strains of caged hens is unlikely to induce persistent pain.

# SECTION IV: WELFARE IMPLICATIONS OF FOOT LESIONS IN CAGED LAYERS.

## Introduction

Laying hens are subject to lesions (hyperkeratosis) in different parts of the foot. The majority of lesions occur at the distal toe pad, with the most severe inflammation involving swelling of the foot pad from pressure from standing on the wire. The aim of this study was to assess the welfare of caged hens with foot lesions compared to a control group of hens without foot lesions using behavioural and histopathology indices.

## Material and Methods

*Hens*

Forty hens (70 weeks of age) selected from a caged flock of 2000 crossbred commercial layers were allocated in pairs to single tier laying cages (45 x 45 x 40 cm) and maintained on a layer ration. Each pair of hens were allocated into one of two treatment groups on the basis of the presence or absence of foot lesions. Treatment 1, n = 20 hens with foot lesions and treatment 2, n = 20 had no foot lesions.

*Video recording of behaviour, viewing video tapes and analyses*

A video recording was made for each pair of hens in each treatment post lay from 1300h-1600h with food and water available *ad libitum.* Data on behaviour were obtained from watching video records and manually keying observations into a hand held micro-computer. The activities recorded were time and bouts of pecking at food, drinking, preening, sitting and number of pecks made at the cage and other birds. Two separate bouts of behaviour were recorded if they were separated by a pause of at least five seconds duration. The incidence of dust bathing, feather ruffling and head scratching were also recorded. SAS linear modelling procedures were used to analyse the effect of foot lesions on the behaviour of the hens.

*Histopathology and immunohistochemical labelling of nerve fibres*

After video recording, all hens were killed by cervical dislocation and toes taken from each treatment group. The toes were fixed by immersion in Zamboni's fixative (Stefanini *et al* 1967) for two to four weeks at 4°C and decalcified for a further 3 weeks. Six toes from each treatment were processed by routine wax-embedding and 5μ m-thick transverse sections stained with either haematoxylin and eosin, or Verhoeff and van Gieson for visualisation of tissue types and any inflammation. Frozen sections of 40-60μm were prepared from the remaining toes and processed either by silver impregnation for general distribution of nerves (Lunam *et al* 1996) or immunohistochemical identification of nerve fibres labelling for substance P as described previously (Lunam 1993).

## Results

### *Behaviour*

Hens with foot lesions showed no significant differences in numbers of bouts of sitting, preening, eating and hen or cage pecks compared to that of hens with no foot lesions. The number of drinking bouts was significantly higher in hens with foot lesions compared to that in hens without foot lesions, P = 0.006, (Table7). The incidence of hen peck bouts in birds with foot lesions was lower compared to hens without foot lesions, though this difference was not statistically significant, P = 0.06.

**Table 7**. Effects of foot lesions (FT) on number of bouts per hour of sitting (SB), preening (PB), hen pecking (HPB), cage pecking (CPB), eating (EB) and drinking (DB). (NFL) is no foot lesions. P = probability value in analysis of variance.

| Treatment | SB | PB | HPB | CPB | EB | DB |
|---|---|---|---|---|---|---|
| FL | 5.2 | 32.7 | 4.5 | 1.9 | 17.6 | 8.8 |
| NFL | 3.8 | 32.2 | 8.9 | 1.3 | 16.7 | 4.1 |
| P | 0.17 | 0.85 | 0.06 | 0.52 | 0.74 | 0.006 |

Hens with foot lesions showed no significant differences in time spent sitting, preening, eating, drinking, head scratching or dust bathing compared to that of hens without foot lesions. Feather ruffling was the only behaviour which differed significantly between the treatments; the incidence of bouts of feather ruffling being significantly higher in hens with foot lesions compared to that of hens without foot lesions (Table 8).

**Table 8.** Effects of foot lesions (FL) on time (seconds) spent sitting (ST), preening, (PT), eating (ET), drinking (DT) and incidence of feather ruffling (FR), head scratching (HS) and dust bathing (DB) averaged over one hour.
P= probability in analysis of variance.

| Treatment | ST | PT | ET | DT | FR | HS | DB |
|---|---|---|---|---|---|---|---|
| FL | 1027 | 1016 | 732 | 146 | 0.25 | 3.7 | 0.9 |
| NFL | 735 | 949 | 683 | 187 | 0.03 | 4.4 | 0.7 |
| P | 0.16 | 0.51 | 0.64 | 0.44 | 0.02 | 0.26 | 0.55 |

### *Histopathology*

Macrophages and small aggregations of lymphocytes were observed in all toes with and without lesions. An inflammatory response, marked with mast cells and eosinophils was more extensive in regions of the toes with lesions than comparable anatomical regions of toes without lesions. Silver impregnation revealed most nerves were associated with the blood vessels near fat pads and tendons. Immunohistochemistry revealed few freely ending nerve fibres labelling for substance P. The distribution and number of these immunolabelled nerve fibres were similar in toes with and without lesions.

## Discussion

These studies examined whether hens with foot lesions exhibited changes in behaviour that may indicate they were in pain. Although both treatments spent a similar average time per hour drinking, hens with foot lesions had significantly more drinking bouts, each bout of shorter duration, than hens with foot lesions. One explanation for the difference in drinking bouts is that the foot lesions become sore when additional pressure is placed upon them during reaching to drink from the water nipple. There was a non-significant trend ($P = 0.16$) for hens with foot lesions to spend more time sitting, suggesting they found it more uncomfortable to stand than hens without lesions. In addition, there was a trend for hens without foot lesions to be more aggressive, as they engaged in more bouts of feather pecking than hens with lesions. Birds with foot lesions had a greater incidence of feather ruffling than hens without lesions. A possible explanation for this is that feather ruffling is a displacement activity that increases with the stress on the hen originating as pain from the foot lesion.

The often extensive inflammatory response in the region of the foot lesions, marked with numerous eosinophils and mast cells, is consistent with inflammation associated with acute and/or consistent pain in mammals. The presence of free nerve endings labelling with substance P indicates that at least some nerves associated with the lesions are likely to be nociceptive, that is, they are capable of transmitting painful stimuli. These data, when considered with our behavioural findings support the suggestion that foot lesions are likely to be painful and thus compromise the well-being of the hens.

# IMPLICATIONS FOR THE EGG INDUSTRY

- The results indicate that feather loss in either the Australian or European strains of caged layers does not decrease the long term well-being of hens, although the degree of acute pain and stress due to feather pecking by cage mates was not assessed. Indeed, the anatomy suggests that although feather abrasion by the cage is unlikely to adversely affect the well-being of the hens, acute removal of the feathers is likely to be painful.
- The incidence of foot lesions in this study was less than 1%. Hens with foot lesions demonstrated histological changes with accompanying behaviours that suggest the lesions are painful and thus decrease the well-being of the hens.
- The European strain is more aggressive towards other hens, as it demonstrated a greater incidence of feather pecking compared to the Australian strain. This finding supports the anecdotal evidence from Industry.

# RECOMMENDATIONS TO INDUSTRY

- Australian egg farmers will need to employ the same husbandry strategies to control feather pecking in European Brown strains as they do for Australian strains.
- A suggested method to improve feather cover in hens toward end of lay is to transfer hens with poor feather cover to the same cages. This may result in improvement of their feather cover and decrease feed costs. These savings need to be balanced against a potential decline in egg production and shell quality as a result of relocation.

# FUTURE RESEARCH

- Using anatomical, physiological and behavioural indices, determine the degree of pain/stress suffered by the hen during the actual removal of the feathers by hen pecking.
- Determine whether pulling of feathers from specific regions of the body is more painful than other regions, that is neck, leg, tail feathers etc. It may be that some feather abrasion by the cage of the more sensitive areas actually protects the hens from acute pain by feather pecking in these areas.
- To determine if feather cover affects productivity, efficiency and profitably of commercially housed layers in winter and summer.
- To determine whether the decline in feather cover with age can be offset by stimulation of the preening gland.
- To examine the effects of relocation of caged hens with poor feather on productivity, efficiency and profitably in winter and summer.
- Assess the prevalence of neuromas in toe stumps of caged layers after trimming; and whether the procedure can be modified, if necessary, to minimise neuroma formation, as we have achieved with beak-trimming.
- To conduct a survey to determine the incidence of foot lesions in the Industry.

# BIBLIOGRAPHY

Alvey DM and Tucker SA (1993) *Proceedings of Fourth European Symposium on Poultry Welfare*, Edinburgh. 242-243.

Appleby MC, Smith SF and Hughes BO (1992) *British Poultry Science* **33**: 227-238.

Blokhuis HJ (1986) A*pplied Animal Behaviour Science*. **16**: 63-67.

Blokhuis HJ (1989) *Applied Animal Behaviour Science*. **22**: 65-73.

Blokhuis HJ and Van Der Haar JW (1989) *Applied Animal Behaviour Science*. **22:** 359-369.

Burger RA, Atuahene YO and Arscott GH (1984). *Poultry Science*. **63**: 997-1002.

Craig JV (1992) *Poultry Science*.**1**: p10.

Duncan IJH, Slee GS, Seawright E and Breward J (1986) *British Poultry Science.* **30**: 479-489.

Gentle MJ and Hunter LN (1990) *Research Veterinary Science.* **50**: 95 -101.

Gentle MJ, Waddington D, Hunter LN and Jones RB (1990) *Applied Animal Behavioural Science.* **27**: 149-157.

Gilbert AB (1965) *Stain Technology.* **40**: 301-304.

Glatz PC (1990) *Australian Journal of Experimental Agriculture*. **30**: 349-355.

Glatz PC (1993) *Final Report to Egg Industry Research and Development Council.*

Koelke KW, Amoss MS and Cain JR (1987) *Poultry Science.* **66**: 397-407.

Kostal L, Cavory CJ and Hughes BO (1992) *Applied Animal Behaviour Science*. **32**: 361-374.

Lee BD, Morrison WD, Leeson S and Bayley HS (1983) *Poultry Science.* **62**: 1129-1132.

Lucas AM and Stettenheim PR (1972) Avian Anat. Agric. Handbk. 362. Integument II. U. S. Government Printing Office. Washington DC. pp. 485-636.

Lunam CA (1993) *Journal of the Autonomic Nervous System.* **44**: 189-196.

Lunam CA and Glatz PC (1993) *Proceedings of the Australian Poultry Science Symposium, Sydney.* **5**:49-52.

Lunam CA and Glatz PC (1995) *Proceedings of the Australian Poultry Science Symposium, Sydney* **7**: 176-179.

Lunam CA, Glatz PC and Hsu Y-J (1996) *Australian Veterinary Journal* **74**: 46-49.

Stefanini M, De Martino C and Zamboni L (1967) *Nature.* **216:** 173-174.

Tauson R (1980) In: Moss R. (ed) *The Laying Hen and its environment*. Martinus Nijhof, The Hagu, pp 269-304.

Tauson R and Jansson L (1988) *Proceedings of the VIII European Poultry Conference*. **2**: 676-680.

Tauson R (1986) *Technical environment for caged laying hens.* Ph.D. dissertation to Swedish University of Agricultural Sciences, Uppsala.

Vestergaard KS, Kruijt JP and Hogan JA (1993) *Proceedings of the Fourth European Symposium on Poultry Welfare*. Edinburgh. 242-243.

Woolford RC, Glatz PC and Hughes RJ (1990) *Proceedings of the Australian Poultry Science Symposium, Sydney*. **2:** 104.