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Human-animal relationships in the laying hens

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Executive summary

The human-animal relationship consists of the behavioural interactions that occur between humans and animals, and the relevant precursors and outcomes of these interactions. One of the main precursors of human behaviour is attitude, and one of the main outcomes of human behaviour in the farming industries is the fear of humans experienced by the farm animals. Animals that are fearful of the stockpeople may experience poor welfare. Furthermore, fear through stress may lower productivity in farm animals. Thus, there are four facets to the human-animal relationship in farming situations: stockperson attitudes; stockperson behaviour; fear of humans in the animals, and the subsequent welfare and productivity of those animals. This project examined each of these facets within the egg industry, with particular attention to the impact of human behaviour on the fear, welfare and productivity of laying hens.

Field work was conducted on Australian and US egg farms. The attitudes and empathy of stockpeople in the egg industry were found to be predictive of their behaviour whilst working. Generally, negative attitudes were associated with more noise being made in the shed, faster speed of movement by the stockperson, more time spent in the shed, less time in certain areas of the shed and less time spent standing still. Positive attitudes and empathy were associated with less noise and less time spent in the shed. In turn, the behaviour of the stockpeople was related to the fear of humans displayed by laying hens during two behavioural tests. The hens displayed less fear of humans in sheds where the stockpeople spent more time standing still, moved more quickly, and expressed more behaviours in close proximity to the birds. In contrast, an increase in fear of humans in the hens was associated with the amount of noise that was made by stockpeople in the laying shed. Surprisingly, fear of humans in the hens was associated with low concentrations of corticosterone, a stress hormone, in the albumen of their eggs, as well as improved egg production. This result cannot be explained, however it may be related to the management style and cleanliness of the laying sheds.

A series of experiments were conducted to further explore the relationship between human behaviour and fear of humans in laying hens. In these experiments, proximity of human contact was shown to be influential in reducing fear of humans in hens, with close proximity of humans resulting in lower fear. The duration of this contact appeared to have little or no impact on fear. Additional contact during rearing resulted in a persistent reduction in fear of humans in adulthood. In addition, the same treatments that reduced fear of humans in the hens were also associated with improved productivity, and a reduction in the plasma corticosterone response to human presence and handling.

From these results it is concluded that the human-animal relationship has important consequences for laying hens in the egg industry, particularly in terms of their welfare. While fear was not negatively associated with egg production in the field study, the experimental study in which fear was manipulated demonstrated that fear of humans can reduce egg production in laying hens. The development of training packages that target the key attitudes and behaviour of stockpeople have the potential to improve the welfare of commercial laying hens by reducing their fear of humans.

It should be noted that this research formed the basis of Lauren Edwards' PhD program, and more information on this research can be found in the thesis if required.

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Introduction

This project examined the human-animal relationship in the egg industry, and the implications of this relationship for the welfare and productivity of laying hens.

The welfare of laying hens in conventional cages is one of the most contentious issues in animal welfare to date. In Australia it is estimated that between 79-83% of laying hens are housed in conventional laying cages (Cransberg and Parkinson, 2007, Runge, 2006). The barren environment and the behavioural restrictions imposed by the cage have caused considerable protest, however the welfare of the hens is influenced by a number of other factors that are not as immediately obvious to the public. For example, the lack of physical exercise combined with the high calcium requirements of egg production can result in osteoporosis, placing the hens at greater risk of bone breakage during the depopulation of the cages (Whitehead, 2004). The birds can also develop abnormal behaviours, such as feather pecking and cannibalism, which pose obvious threats to welfare for the victim involved (Newberry, 2004).

The management and husbandry of laying hens are also important determinants of their welfare, as the hens are totally dependent on the stockpeople. Not only do the stockpeople provide for the physical needs of their animals, but they also interact with their animals behaviourally. The importance of human-animal interactions to animal welfare has been previously demonstrated in other intensive farming situations, such as the pork, dairy and veal industries (Lensink et al., 2000, Waiblinger et al., 2002, Hemsworth et al., 1994a, Hemsworth, 2003). In these studies the behaviour of the stockperson while interacting with his or her animals determined the level of fear of humans that those animals experienced. A high proportion of positive or neutral interactions allowed the animals to habituate to the presence of the stockperson, whilst a high proportion of negative interactions resulted in fearful animals (Hemsworth and Coleman, 1998). Fear is considered an undesirable emotional state that may constitute suffering and reduced welfare (Jones and Waddington, 1992). Fear is a motivator that can override the behavioural patterns of other motivational systems, such as hunger, thirst or reproduction (Murphy, 1978, Jones, 1996, Boissy, 1998), and when the British Farm Animal Welfare Council made revisions to the first Welfare Code of Practice in 1979, ‘freedom from fear’ was one of the five basic rights it advocated (cited in Jones, 1996). These ‘Five Freedoms’ are still in use today, and recognise the importance of reducing fear to improve animal welfare. Fear can elicit just as great a stress response as a physical stressor (Dantzer and Mormede, 1985), and repeated exposure to a fearful stimulus, such as the stockperson conducting routine husbandry on a daily basis, can result in chronic stress for the animal. This stress is indicative of the poor welfare that the animal is experiencing, and has been found to limit the productivity of farm animals (Hemsworth and Coleman, 1998).

Cognitive-behavioural intervention training has been successfully used in the pork and dairy industries to improve the human-animal relationship. This method of training targets the key attitudes that stockpeople have toward working with their animals, and also encourages behavioural change. Because attitudes are the main antecedents of human behaviour, changing stockperson attitudes can be an effective method of improving their behaviour whilst working with their stock. This training method has been shown to resulted in less negative interactions used by stockpeople in handling their animals, lower fear of humans in the animals, and improved productive output (Hemsworth et al., 1994a, 2002; Coleman et al., 2000).

The human-animal relationship has not yet been fully explored in the egg industry. Previous research has shown that fear of humans in commercial laying hens can vary markedly between farms (Barnett et al., 1992). This variation may be due to qualitative differences in the human-animal relationship on these farms. If so, the potential may exist to manipulate this relationship and improve hen welfare by reducing their fear of humans. However, detailed information on the human-animal relationship in the egg industry is currently lacking. Whilst much work has investigated the effects of human behaviour on fear of humans in laying hens, very few studies have been conducted in a commercial setting, and none have included the attitudes and beliefs of stockpeople as a component of the relationship. It is apparent that to fully understand the human-animal relationship in the egg industry, a detailed study of stockperson attitudes, stockperson behaviour, fear of humans in laying hens and their resulting welfare must be conducted in a commercial setting.

Objectives of the project

The aim of this project was to determine the impact of the human-animal relationship on the welfare of commercial laying hens. The key areas of research in this study were:

1. To examine the existence of the human-animal relationship in the current egg industry.

2. To examine the influence of the human-animal relationship on the behaviour, physiology and productivity of laying hens.

If the human-animal relationship is found to influence bird welfare and productivity, the outcomes of this research will be used to develop stockperson training packages to improve the human-animal relationship in the egg industry, with improved outcomes on hen welfare and productivity.

Outline of the report

The following chapter (Chapter 1) describes field research conducted on commercial egg farms in Australia and the US to examine the existence of the human-animal relationship in this industry. This chapter also includes the methodological development of the human behaviour observations, and the development of an attitude questionnaire for stockpeople. Chapter 2 describes an experiment that examined the hypotheses generated during the field study described in Chapter 1. This experiment imposed human handling treatments on laying hens and monitored the resulting behavioural and physiological responses of the hens to further human contact.

The results from Chapter 2 were used to identify some of the key human behaviours that are important determinants of fear of humans in hens. These key human behaviours were used as handling treatments in a large scale laboratory experiment, presented in Chapter 3. This experiment was conducted at the Ohio State University, and was designed to examine the impact of handling treatments on the behaviour, physiology and egg production in laying hens.

A general discussion in Chapter 4 summarises the results of the field and experimental work, and discusses the possible implications of this research for the egg industry.

Methodology

The methods used during this project are described in the methodology sections of each chapter.

Chapter One: Evidence for the human-animal relationship in the egg industry

1.1 Introduction

The human-animal relationship is becoming increasingly important to the welfare of farmed species due to the intensification of farming systems. Within these systems, farm animals are regularly exposed to contact with humans due to husbandry and maintenance routines, and the way that the stockperson interacts with the animals during these routines will determine the way that the animals perceive that stockperson. If the majority of the stockperson's behaviour is aversive to the animals then they will become fearful of humans (Hemsworth and Coleman, 1998). Human contact is unavoidable in intensive housing systems, and whilst many husbandry tasks are becoming automated, human labour is still required. If the animals are fearful of humans then the necessary human contact will evoke a fear response. Fear can be deemed to have a negative impact on animal welfare using several definitions of welfare. Firstly, fear is a negative subjective experience. Secondly, the animal is often prevented from carrying out its desired behavioural response, such as escaping or hiding, and thirdly, fear can elicit a stress response similar to a physical stressor (Dantzer and Mormede, 1985). Chronic physical stressors are well recognised as having a negative effect on the welfare of farmed species, and there is no less reason to dismiss the impact that psychological stressors have on the welfare of these animals. If the stockperson behaves in such a manner that the animals are able to habituate to human presence then the detrimental effects of fear will diminish. However, if the animals are unable to habituate then their fear of the stockperson can develop into a chronic stressor (Hemsworth and Coleman, 1998).

Several studies have examined fear of humans in farmed species and have found large variation between farms in the level of fear of humans displayed by the animals (eg. Lensink et al., 2001, Hemsworth et al., 1981, Waiblinger et al., 2003, Barnett et al., 1992). This variation has been attributed to the differences in human contact that these animals receive.

The way that a person behaves is largely determined by the attitudes of that person, and these attitudes are based on the beliefs that the person holds about the outcomes of performing those behaviours (Ajzen, 1991). Previous research has identified sequential relationships between stockperson attitudes and stockperson behaviour, and their impacts on animal fear, welfare and productivity in several livestock industries (Hemsworth and Coleman, 1998, Breuer et al., 2000, Waiblinger et al., 2002, Hemsworth et al., 2002, Hemsworth et al., 2000, Hemsworth et al., 1994a, Hemsworth et al., 1989a, Hemsworth, 2003, Cransberg et al., 2000). From this work it is clear that stockperson attitudes and behaviours have important consequences for the welfare of farmed animals, and an understanding of these sequential relationships would provide opportunities to reduce fear of humans and improve welfare in these animals.

The egg industry has undergone massive changes in the intensification of its housing systems during the last 60-70 years, and in terms of animal numbers is the biggest animal production industry in the world (Mench, 1992). The human-animal relationship in this industry warrants investigation due to the large numbers of animals involved, and the potential impact of fear on caged animals that cannot escape from a fearful stimulus. Previous work on human-animal relationships with poultry in commercial situations has confirmed the existence of parts of the model of the human-animal relationship (Cransberg et al., 2000, Barnett et al., 1992, Hemsworth et al., 1994b, Graml et al., 2007). Fear of humans was associated with lower productivity in laying hens (Barnett et al., 1992) and broilers (Hemsworth et al., 1994b, Cransberg et al., 2000) on commercial poultry farms. Fear of humans was reduced in free-range laying hens by altering the amount and quality of human contact that they received (Graml et al., 2008), although production was not measured in this study. The only study conducted in the poultry industry that assessed the attitudes of stockpeople was conducted with broilers (Cransberg et al., 2000). This study did not find a relationship between stockperson attitudes and stockperson behaviour, but did find significant relationships between stockperson attitudes and bird behaviour. The author concluded from this result that stockperson attitudes were indeed influencing the way that stockpeople interacted with their birds, but the relationship was not captured by the behaviour variables measured in this study.

This previous industry research provides limited evidence that is consistent with a sequential human-animal relationship in the egg industry (Barnett et al., 1992, Hemsworth et al., 1994b, Cransberg et al., 2000, Graml et al., 2008). To investigate this relationship further, a study of the human-animal relationship was conducted in Australia and the USA. Four aspects of human-animal interactions were examined: stockperson attitudes; stockperson behaviour; fear of humans in laying hens, and hen productivity. The attitudes of stockpeople toward laying hens and their work were assessed using an attitude questionnaire. Stockperson behaviour during routine husbandry tasks was observed for two days in each laying shed. Fear of humans in the laying hens was assessed using two behavioural tests that were designed to assess the hens' avoidance of the researcher. An assessment of the stress that the hens were experiencing was made by collecting a sample of eggs from each shed and analysing the albumen for corticosterone concentrations. Finally, production was assessed by obtaining the productivity records for each flock from the producers. The aim of this study was to examine the between-farm relationships between stockperson attitudes, stockperson behaviour, fear of humans in laying hens and hen productivity on commercial farms in Australia and the USA.

1.2 Methodological development of the attitude questionnaire

1.2.1 Focus groups

Qualitative information on the opinions, issues and concerns of stockpeople who work in the egg industry were required to design the attitude questionnaire. To obtain this information, focus groups were conducted with stockpeople who were encouraged to discuss their work in an informal manner, guided by the researcher. To recruit stockpeople, three large egg farms were contacted and permission obtained from management to recruit their staff. The researcher then attended each farm to discuss the research and recruit stockpeople to attend the focus group, which was held on-farm during the lunch hour at a later date. Thus, three focus groups were conducted, and all stockpeople in each focus group were employed by the same farm. Each focus group consisted of four to seven stockpeople, with a member of farm management present in two of the focus groups.

Each session began with the researcher learning the names of all participants, and providing a brief introduction to the topic of human-animal interactions and the need for focus groups to develop an attitudinal assessment questionnaire. Permission was obtained for the session to be tape recorded and consent forms were signed. The researcher then guided the discussion by asking questions about a series of broad topics, beginning with the daily routine of the stockpeople and their work in the laying sheds. The discussion was then guided onto the more specific topics of the characteristics of the birds, and the way that they respond to human contact. The researcher also had a list of probe questions that addressed specific aspects of the broad topics. These probe questions were used to stimulate discussion if the broad topics did not do so. An example of a broad topic question relating to the working in the laying sheds is 'What is the work environment like in the sheds?' An example of a probe question for the same topic is 'Would you describe the sheds as dusty?' The same list of questions was used for each focus group, and each group was run by the same researcher.

1.2.2 Questionnaire development

Following the focus groups, the tapes were transcribed and the transcriptions examined to identify the underlying issues for stockpeople in the egg industry, how these issues affect what the stockpeople do, and how stockperson behaviour affects the laying hens. That is, what are the beliefs underpinning the way that stockpeople conduct their work? All of the responses to each question were compared and the issues identified. For example, the question ‘How would you describe laying hens?’ obtained the following responses:

- Watching you like they’re mesmerized
- Inquisitive
- Anything moving they'll watch it
- Timid
- They get pretty scared by you reaching into the cage
- They like running away from you
- A lot of them are different
- If you go to open the cage door they won't move their head away
- Some of them are more scared than others
- Most of the hens don't get a lot of human contact, because there's a lot of hens to a few people
- Some are quieter than others
- White hens are by nature more flighty
- Different breeds have different temperaments and habits and things
- Interesting
- Nervous
- Protective
- Dumb
- No one said they were brainy
- An amazing little machine

The underlying beliefs of the stockpeople about laying hens were thus related to the birds being inquisitive, afraid of humans, variable in their response to people either individually or as a strain, unintelligent, protective (of their eggs), interesting, and machine-like. Statements were then devised based on these beliefs, such as ‘Seeing people is the highlight of a hen’s day’, and ‘Hens are easily scared of people’. These statements were created for all of the issues and beliefs identified from the transcripts of the focus groups. A final list of statements was selected for use in the questionnaire, and this list was divided into three sections. The first section was labeled ‘Working with laying hens’, and consisted of statements about working with the hens and about the husbandry tasks in the laying sheds. The second section was labeled ‘Characteristics of laying hens’, and consisted of positive and negative statements about laying hens. The third section was labeled ‘Interacting with laying hens’, and consisted of statements about interacting with the hens and how they responded to humans.

The stockpeople were asked to indicate whether they agreed or disagreed with each of the statements using the Likert scale, which consists of five options: strongly agree, agree, neither agree or disagree, disagree, and strongly disagree. By using a variety of statements relating to a particular topic, consistent beliefs relating to that topic could be recognized and the attitude toward that topic inferred (Hemsworth and Coleman, 1998). Thus, the beliefs that the stockpeople held in relation to statements about the hens and their work were used to assess their specific attitudes toward the hens and their work.

Calculation of attitude subscales

There were often several different stockpeople observed in a shed, however there was generally one stockperson responsible for the majority of work in each shed. The questionnaire was administered to that stockperson. If more than one stockperson was responsible for a shed then the questionnaire was administered to both. This resulted in a sample size of n = 31 stockpeople for the 29 sheds studied.

The large number of questionnaire items could not be reduced to a more manageable size using a principal components analysis, because the number of items in the questionnaire was greater than the actual sample size. Thus, to reduce the questionnaire data to a manageable size the questionnaire items were sorted into subscales with similar content. This resulted in ten subscales of items, and the items in each subscale were summed to create a single value for each subscale for every stockperson. All of the items in a subscale were significantly correlated with the subscale total, and all subscales had a Cronbach's alpha score above 0.7, indicating that the items in each group were measuring the same attitude dimension. A list of the items that were summed to create each subscale are presented in Tables 1 – 4. The author's subjective labelling of each subscale based on semantic content of the items is also included in these tables.

Table 1 presents some of the behavioural attitudes that stockpeople hold about their work. The majority of items in these subscales relate specifically to the behaviour of the stockperson whilst working in the laying shed, as the attitudes that the stockpeople have about the outcomes of these behaviours may alter the way that they conduct their work. The questionnaire asks stockpeople to agree or disagree with statements about certain aspects of their work, and the response that stockpeople provide is an assessment of whether their attitude to the outcome of that behaviour is positive or negative. For example, items in the behavioural attitude subscale 'Sensitivity of stockperson' relate to the stockpeople's awareness of the impact that their behaviour has on the behaviour of the birds. Stockpeople who agree with items in this subscale, such as 'I will often sit or stand and just watch the hens' and 'I should act carefully around laying hens so as not to scare them', are indicating that they agree that the outcome of performing these behaviours is important, and that they actually perform these behaviours, or intend to perform them, whilst working with the hens.

Table 2 and Table 3 present the general beliefs that stockpeople hold about laying hens. Attitudes toward specific objects are formed from the beliefs that people hold about that object, and it is appropriate that the beliefs of stockpeople about the hens be assessed.

Table 4 presents the items that formed the dispositional subscale 'Empathy'. It should be noted that the variable 'Empathy' is not actually an attitude, as it is assessing the stockperson's self, rather than an external object or the outcome of a behaviour. Coleman et al. (1998) describes empathy as the "capacity to vicariously experience the emotions of another". Empathy has been previously related to stockperson behaviour in the pork industry (Coleman et al., 1998), and it was considered appropriate to include in the present study.

Table 1 The seven attitude subscales that relate to the behavioural attitudes of the stockpeople observed during the field work, the associated Cronbach's alpha score for each subscale, the items that were summed to create the subscale total, and the author's interpretation of the attitude subscales

Attitude subscale	Cronbach's alpha	Items in the attitude subscale	Description of the behavioural attitude
Insensitivity of stockperson	0.75	Yelling at the birds quietens them down Moving birds is dirty work I am quite assertive at work Laying hens aren't affected by the way they are treated Productivity is determined by how much food and water the hens have access to Giving the birds a fright doesn't have any long term effects on production If the flock gets a bad start at the hatchery then nothing I do will change that It doesn't matter much what I do because some flocks are more flighty than others How quickly I work doesn't affect the layers' production Human contact is the highlight of a laying hen's day A laying hen cannot hurt a human I often start cleaning quickly but slow down by the time I get to the end of the shed It is important to ensure that all dead birds are collected every day	The stockperson is partially aware that his or her behaviour can influence the hens, but does not perceive this to have any negative effects. The stockperson is unlikely to alter his or her behaviour to minimise the fear experienced by the birds.
Unpleasantness of job	0.87	I am always rushing when I check the egg belts Removing dead hens is the worst job on the farm Laying hens are frustrating to work with It is difficult to care for laying hens I find the shed too noisy I let the layers know who's boss to avoid being pecked I only talk to the birds to shut them up I cough a lot when I am cleaning I work quickly on a boring job to get it out of the way I will often make a noise just to see the response of the birds The smell in the shed bothers me Removing dead hens is someone else's job I find working with layers boring	The stockperson finds the work unpleasant, and dislikes certain aspects of the job.
Stockperson enjoys job	0.79	I don't mind the dust in the shed I am happy with the amount of walking I have to do in the shed Laying hens are a pleasure to work with Laying hens are easy animals to work with	The stockperson enjoys the work, and is not concerned with the negative aspects of the working in the shed, such as the dust levels.

		Laying hens are easy to manage I like handling live birds I whistle when I am having a good day I prefer to work in the sheds rather than outside.	
Diligence	0.76	I clean slowly to avoid stirring up too much dust A good work ethic is required to work with laying hens The first thing I do every day is check that the automatic systems (food, water, egg collection) are working properly I put the same amount of care into interesting work as I do with repetitious work I try to make the hens as comfortable as possible I am careful to stick to a regular routine If I work slowly and steadily I'm not tired by the end of the day I am very thorough in my work	The stockperson considers their work important, and puts an emphasis on doing the work thoroughly and correctly.
No job control	0.73	I don't have much control over what I do in the shed I have no input on how my job is done I just do what I am told when working in the shed If I don't agree with something I don't do it I just do what I'm told I need to work quickly to get all the jobs done	The stockperson feels that he or she has no control over how the work is done, and is simply told what to do by management.
Job control	0.70	Management listens to my suggestions I am an important member of a team	The stockperson feels valued, and able to contribute to how his or her job is done.
Sensitivity of stockperson	0.74	I will often sit or stand and just watch the hens I should act carefully around laying hens so as not to scare them Birds are easier to handle if they aren't scared Older hens are easier to work with than pullets I notice differences in the way laying hens respond to me I stop what I am doing if I hear a change in the noises that the birds are making So long as I'm quiet I don't disturb the birds It is important to keep a regular daily routine when caring for layers I must be careful when handling the birds so that they will be calmer in the future A calm bird is more easy to handle	The stockperson is aware that his or her actions influence the birds, and that care must be taken to minimise the fear that the birds experience due to the stockperson.

Table 2 The questionnaire items that were summed to create the general attitude subscale 'Negative General Attitudes' for the stockpeople observed during the field work, the associated Cronbach's alpha score for this subscale, and the author's interpretation of this attitude subscale

Attitude subscale	Cronbach's alpha	Items in the attitude subscale	Description of the general attitude
Negative general attitudes	0.81	Layer hens are dirty animals Layer hens have an ugly appearance Layer hens are greedy Layers hens are made out to have more feelings than they really do Layer hens are unfriendly Layer hens are cruel Layer hens panic for no reason Layer hens are noisy animals Layer hens are smelly animals Layer hens are easily frightened Layer hens are frightened of humans Layer hens don't remember like humans Layer hens don't feel pain like humans	The general beliefs that the stockperson has about laying hens are negative

Table 3 The items that were summed to create the general attitude subscale 'Positive General Attitudes' for the stockpeople observed during the field work, the associated Cronbach's alpha score for this subscale, and the author's interpretation of this attitude subscale

Attitude subscale	Cronbach's alpha	Items in the attitude subscale	Description of the general attitude
Positive general attitudes	0.83	Layer hens are entertaining to watch Layer hens are intelligent animals Layer hens require respect Baby chickens are cute Layers hens have feelings Layer hens are sensitive Layer hens like humans Layer hens are friendly towards people Layer hens are curious animals	The general beliefs that the stockperson has about laying hens are positive

Table 4 **The items that were summed to create the personality subscale 'Empathy' for the stockpeople observed during the field work, the associated Cronbach's alpha score for this subscale , and the author's interpretation of this dispositional subscale**

Attitude subscale	Cronbach's alpha	Items in the attitude subscale	Description of the personality subscale
Empathy	0.81	It is kinder to handle the birds gently I try to understand laying hens by imagining how things look from their point of view I feel bad if the hens go without food or water When I see a contented laying hen I feel good	The stockperson is capable of feeling a similar subjective state to that of the hens, and is concerned with the comfort of the hens.

1.3 Methods

1.3.1 Farm Recruitment

Data were collected at 20 Australian sheds and 9 US sheds between March 2005 and June 2006. The US farms were studied over a 5 week period (August – September 2005), visiting two sheds per week. Contact details for egg farms were obtained from public directories and through industry contacts. An introductory letter was sent to each of the farms explaining the study, and these farms were then called a week later to discuss their participation.

Farms were chosen on the basis that they used a conventional cage system and were willing to participate in the study. The selection criteria initially involved only farms that used enclosed, climate-controlled sheds, but this was later changed to include the older style open-sided sheds due to a lack of participants. All possible attempts were made to visit farms whilst the birds were between 38 – 60 wks of age, as this would avoid the intensive period of peak production for the birds, during which the additional stress of undergoing behavioural testing may have negatively impacted the hens. Several farms were unable to be visited while the birds were at this age due to time constraints, particularly in the US sheds, and thus the bird ages that were included ranged between 28 and 105 weeks of age. A description of the each focal shed, including the age of the birds, is presented in Table 5.

The majority of egg farms visited in the study used more than one laying shed. Each shed was considered a separate unit if a distinct team of stockpeople maintained it; therefore more than one shed could be studied at a farm if there were different staff maintaining it. This was possible at four farms, allowing the sample size to increase by eight sheds.

Data were collected at each shed over a three day period. The behavioural tests were administered to the birds and an egg sample was collected on the first day. The following two days were spent observing the human animal interactions that occurred in the laying shed. At the end of the three day period the attitude questionnaire was administered to the main stockpeople observed in each shed. Where possible the productivity records for the entire laying life of the flock were obtained.

Table 5 Descriptions of the physical parameters of the sheds studied in the field work

Shed No.	Country	Aisle length (m)	No. of birds in shed	No. of stockpeople observed	Age of birds (wks)	Strain of birds
1	Australia	86.4	14000	5	50	ISA brown
2	Australia	84.4	12300	4	57	Hyline brown
3	Australia	74.0	24000	6	57	ISA brown
4	Australia	30.4	2500	2	48	ISA brown
5	Australia	38.5	1320	2	48	Hyline brown
6	USA	134.4	96000	2	26	Hyline White 36
7	USA	174.0	125000	3	49	Hyline White 36
8	USA	108.0	40500	2	48	Hyline White 36
9	USA	174.4	183500	2	74	Hyline White 36
10	USA	174.4	183500	3	29	Hyline White 36
11	USA	109.8	40500	1	103	Hyline White 36
12	USA	137.3	81000	1	102	Hyline White 36
13	USA	134.0	96300	1	76	Hyline White 36
14	USA	177.0	107500	2	106	Lohmann
15	Australia	85.1	22016	6	55	ISA brown
16	Australia	41.6	4032	1	50	Hyline brown
17	Australia	54.3	21952	4	36	Ingham Hisex
18	Australia	75.8	29430	1	48	Hyline brown
19	Australia	128.7	108108	5	69	ISA brown
20	Australia	129.4	121968	6	66	ISA brown
21	Australia	93.0	67500	5	65	ISA brown
22	Australia	93.0	67500	10	63	Hyline brown
23	Australia	93.0	67500	5	42	Hyline brown
24	Australia	93.0	67500	8	43	Hyline brown
25	Australia	74.5	21888	4	51	Hyline brown
26	Australia	83.0	68320	9	52	ISA brown
27	Australia	128.7	116424	5	62	ISA brown
28	Australia	128.7	116424	4	60	ISA brown
29	Australia	84.3	22016	4	90	ISA brown

1.3.2 Development of stockperson ethogram and observation methods

Observation method

A stockperson ethogram was developed to measure the main stockperson behaviours that occured in laying sheds. To become familiar with the husbandry tasks and routines carried out by stockpeople, three visits were made to commercial egg farms to observe stockpeople working. During these visits the different tasks and the time spent in the aisles were both recorded. It was soon realised that recording behavioural observations on a data sheet whilst using a stopwatch involved looking away from the stockperson, and this meant that the researcher was unable to constantly observe the stockperson, thus potentially missing some behaviours. A data collection method was required that did not involve the researcher looking away from the stockperson to record the data.

A portable data logger, such as the Noldus Observer, was not deemed a practical solution as the data logger required all possible behaviours to be coded into the logger prior to observations beginning. Due to the large variation in the types of tasks performed by different stockpeople, as well as between-farm variation in the equipment and structure of the laying sheds, it was likely that human behaviours would be observed that had not already been coded into the data logger and would need an additional means of recording them. It was decided that making verbal observations into a tape recorder would be a more thorough method of recording stockperson behaviour.

Stockperson behaviours measured during observations

Stockperson behaviour was observed for two days in each shed. During this time the researcher remained inside the shed and verbally recorded the identity and the time of entry for every stockperson that entered the shed. The researcher then followed the stockperson at a distance of about 10 m and made verbal observations into a small handheld tape recorder. The observations consisted of using a stopwatch to record different types of behaviour that the stockperson performed whilst in the shed. The specific behaviours that were recorded are described in more detail below, and are listed in Table 6. The frequency of behaviours was recorded using a 5 s bout interval, where all behaviours were timed and counted in 5 s bouts, which were then rounded up. For example, a behaviour that lasted 4 s would be recorded as occurring once, whilst a behaviour that lasted 12 s would be recorded as occurring three times.

In addition, the researcher also recorded the amount of time that each stockperson spent in the aisles ('Time in aisle'), at either the start or end of the shed ('Time at start of shed'), at the end of each aisle without walking the entire length of the aisle (such as when maintaining equipment, 'Time at ends of aisles'), and the time spent standing stationary in the aisles ('Time stationary'). This allowed the average speed of movement to be calculated by dividing the amount of time spent walking along the aisles by the length of the aisle ('Av SOM', m/s). The fastest and slowest average speeds recorded for each shed were also included in the analysis (‘Min SOM' and 'Max SOM').

Analysis of stockperson behaviour observations

The verbal tape recordings of the stockperson behaviour observations were transcribed by hand at a later date. During the observations the researcher recorded the duration of each of the main stockperson behaviours and noted the time when the stockperson moved position in the shed, however there were often occasions when the stockperson was moving too quickly for the times to be recorded for every behaviour. On these occasions the researcher simply listed each behaviour verbally as it happened, and used a stopwatch to time the duration of these behaviours at a later date whilst replaying the tape recording. Thus, each verbal recording of stockperson observations was listened to twice: once to transcribe the tape, and again to time the behaviours in the transcription that weren’t timed during the observations.

The tape transcriptions were then analysed and the duration and frequency of each observed behaviour was counted for all human activity that was observed in the focal shed during the observational period. Due to the large number of different stockperson behaviours observed, all behaviours were grouped on the basis of their proximity to the birds, resulting in the following six categories of behaviour. A list of all the behaviours recorded and how they were grouped together is presented in Table 6. These categories are described in more detail below.

Visual interactions

Behaviours that occurred in the aisles without the stockperson approaching the cages were labelled ‘Visual’ interactions. Examples of these behaviours are: standing stationary in the aisle; pushing or pulling objects such as floor sweepers; sweeping or scraping the floor, and bending down or crouching.

Noise

Any noise made by the stockperson whilst in the shed was recorded and labelled as ‘Noise’. Behaviours that caused noise in the shed were: yelling by the stockperson; cleaning with an air hose or leafblower; dropping metal objects on the concrete floors, and banging equipment during cleaning and maintenance.

Cage approaches

Behaviours that involved the stockperson or an object the stockperson was holding approaching the cages were labelled ‘Approach’. A behaviour was deemed an ‘Approach’ if the stockperson specifically focused on a single cage without touching it, or if their hand or an object they were holding approached within the width of the feed trough attached to the front of the cage. This distance ranged between 10 and 19 cm due to variation in the width of feed troughs between farms. Examples of these behaviours are: close visual inspection of a cage or feed levels in the feed troughs; the handle of a broom or shovel approaching the cages whilst cleaning; shining a torch into the cage, and placing a hand or object over or under the cages.

Cage contacts

Behaviours that involved the stockperson, or an object that the stockperson was holding, contacting any part of the cages was labelled a ‘Contact’. Examples of these behaviours are: manipulating cages or cage doors; touching the cage front, feed trough or egg belt, including eggs; standing on the feed troughs, and placing objects in the feed trough.

Cage entries

Behaviours that involved any part of the stockperson, or an object that the stockperson was holding, entering the cage were labelled ‘Entry’. Examples of these behaviours are: placing the hands in the cage to remove dead birds or assess sick birds; repairing parts of the cage such as the drinking nipples, and inserting sticks into the cage to roll eggs down to the egg catchers.

Handling birds

Behaviours that involved touching or handling the birds were labelled ‘Handle’. Examples of these behaviours are: removing birds from the cage to inspect them; pushing birds out of the way to access parts of the cage, and catching loose birds that had escaped from their cages.

Table 6 The specific stockperson behaviours that were included in each behavioural category

General behaviour category	Specific stockperson behaviours	
Visual	Manipulate clothing Manipulate object Pause Push object Carry object Kick object	Sweep Crouch Turn around Bend down Drop object
Noise	Noise Loud noise	Yell
Approach	Close visual inspection of cage Throw object onto manure belt Hand approaches cage Torch use	Face over feeder Object approaches cage Object over cage Object under cage
Contact	Contact feeder Lean on feeder Manipulate egg Object on egg belt Object in feeder Hand in feeder Hand under feeder Hand on rail Stand on feeder Stand on rail	Stand on cage Hand on cage Manipulate cage Bang cage Kick egg belt Kick feeder Contact cage Contact feeder with object Contact cage with object
Entry	Insert object into cage Insert one hand into cage	Insert both hands into cage
Handle	Touch bird	Handle bird

Units of measurement

The total number of instances of each type of behaviour observed for all stockpeople over the two day observation period was summed and then divided by the total number of cages in a single tier in all rows of cages in a shed. The number of cages in a tier was the unit chosen as the bird behaviour observations were made on a per cage basis, and the 'per cage' unit was considered the most relevant to the birds. This allowed the behavioural observations to be converted into a standard unit (behaviours / cage) that could be compared between sheds of differing sizes, and resulted in the variables 'Visual / cage', 'Noise / cage', 'Approach / cage', 'Contact / cage', 'Entry / cage' and 'Handle / cage'. The total number of behaviours that involved the stockperson being in close proximity to the birds (cage approaches, cage contacts, cage entries and bird handling) were summed to create the variable 'Near Cage / cage'. The duration of time that the stockperson spent in each area of the shed was also converted into the units 'seconds in each area per cage', creating the variables 'Time at start of shed / cage', 'Time in aisle / cage' and 'Time in Ends of Aisles / cage'. Other variables calculated were the time spent stationary in the aisle (Time Stationary / cage), and the total number of behaviours that occurred (Interactions / cage).

In summary, several aspects of stockperson behaviour were recorded. These aspects were the specific behaviours performed by the stockpeople, composite variables created by summing the behavioural categories, the amount of time spent in certain areas of the shed, the amount of time spent standing stationary, and speed of movement. These variables are summarised in Table 7.

Table 7 A summary of all stockperson behaviours recorded during the two-day observation period in each laying shed

Type of observation	Name of variable	Description on variable
Specific stockperson behaviours	Visual, Noise, Approach, Contact, Entry, Handle	Described in Table 6
	Near Cage	The total number of behaviours that occurred near the cages (the sum of Approach, Contact, Entry and Handle)
	Interactions	The total number of specific stockperson behaviours that occurred (the sum of Visual, Noise, Approach, Contact, Entry, Handle)
Time spent in certain areas of the shed	Time at start of shed	Total time spent at the start or end of the shed (s)
	Time in aisles	Total time spent in the aisles (not including time spent in the ends of the aisles) (s)
	Time n ends of aisles	Total time spent in the ends of the aisles, without walking all of the way along the aisle (s)
	Time stationary	Total time spent standing stationary in the aisles (s)
	Total secs	Total time spent in shed (a sum of the time spent at the start of the shed, in the aisles and in the ends of the aisles) (s)
Speed of movement	Average SOM	The average speed of movement when walking along an entire aisle (m/s)
	Minimum SOM	The minimum value for the average speed of movement (m/s)
	Maximum SOM	The maximum value for the average speed of movement (m/s)

Individual stockperson behaviour observations during bird inspections

In order to study the relationship between a stockpeople's attitude and their behaviour toward the birds, the behaviours of individual stockpeople were correlated with their responses to the attitude questionnaire. This comparison was made for all of the behaviours observed for an individual over the two-day observation period (referred to as 'Total behaviours'), and all of the behaviours observed for an individual during the single task of bird inspections (referred to as 'Inspection behaviours'). The task of bird inspections was chosen because it was a common task observed in every laying shed, and thus was a common task that could be compared between stockpeople in different laying sheds. The additional variable 'Total behaviours' was included as a more encompassing measure of stockperson behaviour in the laying shed, as it included work that did not involve directly interacting with the birds, such as cleaning. The variables included in the analysis were the same as for the observations of all stockpeople, and included the behavioural categories described above, speed of movement, time spent in certain areas of the shed and time spent stationary in the aisles.

1.3.3 Behavioural Tests - Approaching Human Test

The avoidance behaviour of an animal to an approaching stimulus is considered indicative of the amount of fear to that stimulus. The Approaching Human Test (AHT) assesses the behavioural response of an animal to an approaching human, and was used by Hemsworth and Barnett (1993) to assess fear of humans in caged laying hens on commercial farms. This test was adapted for use in the current field study by adding an additional cage approach. This step was added to assess whether the avoidance behaviour of the birds changed with varying stimulus intensity. Thus, it should be recognised that the avoidance behaviour measured in this test also incorporated an element of approach behaviour, as the birds may have moved toward the researcher as she stepped away from the cage and the stimulus intensity decreased. However, the actual variable being measured was the number of birds that were present at the front of the cage, and it was presumed that fewer birds at the cage front was indicative of greater avoidance behaviour and thus greater fear of the researcher.

For the purposes of the behavioural tests, every tenth cage on the second tier on the right hand side of each aisle was flagged as a focal cage. The researcher walked along the aisle and stopped at a distance of one cage from the focal cage. Her hands were kept in her pockets at all times. The researcher waited in this position for 5 s before commencing the test to allow the birds to make visual contact. This avoided a startle response when the test began. During the test an assistant would verbally notify the researcher at 5 s intervals using a stopwatch, and recorded the results.

After the initial 5 s the researcher stepped sideways, directly in front of the focal cage but on the opposite side of the aisle, and stayed in this position for 5 s. During this 5 s period the researcher assessed avoidance behaviour by counting the total number of birds that placed their head or beak in the front 5 cm of the cage. Any birds that put their head through the cage front during this period were also counted. The assistant verbally notified the researcher at the end of the 5 s period, at which point the researcher made a point count of the number of birds still at the cage front. The researcher then verbally relayed the following information to the assistant

- The total number of birds that placed their beaks in the front 5 cm of the cage during the 5 s period ('Maximum Heads')
- The number of birds with their beaks in the front 5 cm of the cage at the end of the 5 s period ('Point Count')
- The number birds that placed their heads out of the cage during the 5 s period ('Heads Out')

The researcher then stepped forward so that her torso touched the feed trough of the focal cage. The researcher remained in this position for another 5 s period, and repeated the same measurements of avoidance behaviour. The researcher then stepped back to the opposite side of the aisle and repeated the process. This resulted in the researcher conducting four 5 s counts of birds in the focal cage (two 5 s periods close to the cage, and two 5 s periods on the opposite side of the aisle). The test created 12 variables for each focal cage: Maximum number of heads, a point count, and the number of heads out, all recorded every 5 s for 20 s. The researcher then moved along the aisle and repeated the test until all focal cages had been tested

A principal components analysis was used to reduce the number of variables obtained from the Approaching Human Test to a manageable number by identifying a small number of components, or groups of variables, that were statistically interrelated. The Approaching Human test had been conducted 2844 times over the entire 29 sheds studied, resulting in a large sample size with which to conduct the analysis. From the twelve variables obtained during the Approaching Human Test, the principal components analysis identified two components of behaviour, which accounted for 82% of the variation in avoidance response. The first component was composed of the variables 'Maximum Heads' at 5 s, 10 s, 15 s and 20 s, the 'Point Count' at 5 s, 10 s, 15 s and 20 s, and 'Heads Out' at 5 s. These nine variables were combined into a single factor score that represented the first behavioural component, and was labelled the 'Forward Score' (Cronbach's alpha = 0.94). The second component was composed of the variables 'Heads Out' at 10 s, 15 s and 20 s. These variables were combined into a single factor score that represented the second behavioural component, labelled the 'Heads Out Score' (Cronbach's alpha = 0.86). A high value for either score indicates a greater number of birds at the cage front or with their heads out during the Approaching Human Test and thus indicating less fear of the researcher.

1.3.4 Behavioural Tests - Stroll Test

The Stroll Test was adapted from a similar test used by Cransberg (2000) to assess fear of humans in broiler chickens. The test was adapted for use with caged laying hens, and involved filming the response of the hens to the researcher walking along the aisles holding a hand-held video camera. Due to the low light levels used in commercial laying sheds, all video footage was filmed in infra-red. The test was administered in one movement throughout the shed, moving along each aisle once. The video camera was held in the right hand, level with the edge of the feed trough and filming the cages ahead of the researcher on the second tier. The camera was held at such an angle that the distance to the first bird with its head out was able to be recorded, with a field of view of at least four cages ahead of the researcher. The angle of the camera was such that the cage divisions could be easily seen during film playback, allowing the cages to be counted during film analysis. During filming the researcher moved slowly through the shed, at a standard speed of one step per s, and filmed the entire second tier of cages on the right–hand side of each aisle. This was the same side of the aisle that the Approaching Human Test had been administered.

During subsequent film analysis the film was paused every 5 s and two measurements made. First, the number of cages between the researcher and the first bird with her head extended out of the cage was counted. As the width of the cages had previously been measured the distance from the researcher to this bird could be calculated. This variable was labelled 'Withdrawal Distance', and measures the distance at which the hens would let the researcher approach before withdrawing their head back into the cage. A greater value for the 'Withdrawal Distance' indicates a greater fear of the researcher. Secondly, the number of birds with their heads extended through the front of each cage was counted for a distance of four cages ahead of the researcher. Only four cages were counted due to low visibility after this distance. The number of heads out in the first four cages was summed, converted to a proportion and then divided by the length of the four cages. This allowed the proportion of birds with their heads out per m distance to be calculated, creating the variable 'Proportion of heads out / m'. A greater value for the 'Proportion of heads out / m' indicates lower fear of humans. This standardised unit was created to account for the different cage widths used between farms.

1.3.5 Corticosterone analysis of egg albumen

A physiological assessment of stress levels in laying hens was required to complement the behavioural data collected during the Approaching Human Test and the Stroll Test. The analysis of corticosterone concentrations in egg albumen provides a simple and non-invasive method of assessing stress hormones in laying hens, and does not require the additional labour and equipment needed when collecting plasma samples.

The analysis of egg albumen for corticosterone content using competitive protein binding radioimmunoassay is a method that was developed relatively recently, and has been used to assess basal corticosterone concentrations in laying hens (Downing and Bryden, 2003, Downing and Bryden, 2002). Egg albumen is formed over a 3-4 hrs period, and during this time the circulating free plasma corticosterone is able to infuse the albumen. The concentration of corticosterone in the egg albumen is indicative of the concentration of corticosterone in the plasma (Downing and Bryden, 2002), and this method was considered suitable for assessing the basal stress levels of laying hens in commercial situations.

A sample of eggs was collected from each shed. Four eggs were collected from four locations along one side of each aisle (one from each end and two from the middle), prior to commercial egg collection starting. Thus, the size of the egg sample varied between sheds of different size, as larger sheds had more aisles than smaller sheds. The eggs were labelled with the position they were collected from and refrigerated until they could be separated. When the eggs were separated, the albumen was weighed and then poured into a plastic centrifuge tube and frozen at – 20 C. The frozen samples were later analysed for corticosterone content by a third party. The results of these assays were used to calculate the average albumen corticosterone concentration (ng/ml) for each of the study sheds, resulting in the variable 'Corticosterone concentration'.

During the process of separating the eggs and removing the albumen, the weight of the whole eggs prior to separation was recorded. This allowed the average egg weight to be calculated for each laying shed, creating the variable 'Egg Wt'. This variable was included in the analysis of hen productivity.

Due to the quarantine restrictions imposed on raw egg product being imported into Australia, the albumen samples from the US could not be analysed. A commercial laboratory that could conduct the same analysis was unable to be located, and the US albumen samples were abandoned.

1.3.6 Assessing productivity

Productivity records were obtained at the conclusion of the study. The records were unavailable for two flocks, and the records for a further two flocks were excluded from the analysis due to missing data. This resulted in a sample size of n = 25 for the productivity data.

The peak rate of lay was determined for each flock and labelled 'PHDP' (Peak Hen Day Production). The age that each flock reached peak production was recorded (Age at PHDP), as was the number of weeks that the flock remained within 2% and 5% of peak production (PHDP2% and PHDP5%, respectively). A high value for PHDP2% or PHDP5% indicates that the flock were sustaining a high rate of lay over a number of weeks. The final production variable obtained from the productivity records was the cumulative mortality rate at 29 wks of age (Mortality). Mortality was measured at 29wks of lay as this was the age of the youngest flock assessed, and mortality data could not be obtained for these birds at a later age.

1.3.7 Procedure for assessing each laying shed

In summary, three days were spent assessing each laying shed. The first day was spent collecting the egg samples and administering the behavioural tests to the laying hens. The following two days were spent observing the stockpeople performing routine husbandry procedures in the shed. At the end of the third day the attitude questionnaire was administered to the participating stockpeople and the productivity records obtained for the flock. All farms were briefed on the results at the conclusion of the study.

The first activity that was undertaken upon entering the shed was to mark the focal cages. This was done by numbering the floor next to every tenth cage with chalk, counted from the first cage in the second tier of the first aisle. On some of the larger farms, every twentieth cage was marked to avoid an excessive number of focal cages, and the requisite behavioural tests associated with them. All of the fully enclosed sheds included in this study had at least three tiers of cages, and the second tier was selected as it was a common tier between different sheds. The number of tiers in the laying sheds varied between three and eight tiers of cages. Testing fear of humans in the top and bottom tiers was avoided as these tiers display the greatest fear response to humans (Hemsworth et al., 1993). The second tier is also the most easily accessible when administering the behavioural tests to the hens. Two of the smaller sheds contained a single tier only, and so this was the tier assessed.

After the focal cages were marked, the egg samples were collected and the behavioural tests administered. Only one side of each aisle was assessed to avoid exposing untested birds to human contact prior to testing. On the majority of farms, the Stroll Test was administered in the morning prior to the Approaching Human Test (AHT), as the Stroll Test is the shorter of the two tests. However, in the larger and more remote sheds ($n = 8$) the AHT was administered first. This avoided the assistant required for the AHT having to wait for up to two hours while the Stroll Test was completed. The order in which the tests were administered had no significant ($P < 0.05$) effect on bird behaviour during the tests.

Once the behavioural tests were complete, additional measures of the shed and cage structures were conducted. These included measuring the light levels (lux) at the cage door of each focal cage, cage dimensions, aisle length, the number of birds per focal cage and a brief sketch of the floor plan in the laying shed. These data were later used to calculate distances and proportions, as well as being directly included as variables in the analyses (shed parameters).

During the second and third day in each shed, the researcher remained inside the shed during working hours and waited for stockpeople to enter the shed. Human behaviour observations were made as per the description in Section 3.3.2. The researcher left the sheds during meal breaks, as it was unlikely that stockpeople would be entering the sheds during this time. At the end of the third day the questionnaire was administered, and the production records obtained.

1.3.8 Statistical analysis

This study resulted in a substantial number of variables, particularly in relation to the measurement of stockperson behaviour. These data will be briefly summarised to clarify the measurements made. Stockperson behaviour was measured on three levels. The first level included all behaviours that were observed for all stockpeople over the entire two-day observation period. These data were used to determine the relationships between stockperson behaviour and hen behaviour. The second level included all of the behaviours observed for an individual stockperson over the two-day observation period. These data were used to determine the relationships between stockperson attitudes and stockperson behaviour. The third level included all of the behaviours observed for an individual stockperson during a standard task (inspecting the birds). These data were also used to determine the relationships between stockperson attitudes and stockperson behaviours whilst all individuals were performing the same task.

All variables were checked for normality and transformed where required. If normality could not be corrected using a transformation then the variable was not transformed, and the untransformed data were used in the analyses. A substantial proportion of the stockperson behaviours were skewed, and the following transformations were made to correct normality. In general, the stockperson behaviour measured for individuals during bird inspections were transformed using a square root transformation, whilst the total behaviours for all stockpeople and the total behaviours for individual stockpeople were transformed using log transformations. Not all variables in an analysis were transformed, and all transformed variables can be identified by the type of transformation in parentheses following it, either (Log) or (SqRt).

Both stockperson and hen behaviour showed substantial variation between Australia and the US. Independent samples t-tests confirmed that significant ($P < 0.05$) differences existed between the two countries for 50% of shed parameters, 69% of stockperson behaviour variables, 100% of hen behaviour variables and 50% of the production variables. The Australian stockpeople spent more time in the sheds and interacted with the birds more often than the stockpeople in the US. Also, the Australian birds displayed markedly less fear of humans than the US birds, the US laying sheds were substantially larger, and hens in the US laid less eggs with a lower egg weight.

Due to the significant differences between countries described above, partial correlation analyses were conducted with country partialled out to determine relationships between stockperson attitudes, stockperson behaviour, hen behaviour and productivity. Stepwise linear regression analyses were conducted to further explore these relationships. The variables included in the regression analyses were selected on the basis that they correlated with the dependent variable with a significance of $P \leq 0.10$. Due to the small sample size, the criterion for the inclusion of a variable in the final regression model was set at $P = 0.10$. As the large differences in bird behaviour and stockperson behaviour that were observed between Australia and the USA were likely to be at least partially explained by differences in bird strain and shed size, the variables 'Country' and 'Strain' were included in the regression analyses using dummy-codes: Country was recoded as Australia = 1 and the US = 2; bird strain was recoded so that brown strains = 1 and white strains = 2. These variables were included to account for any variance in bird behaviour that were due to country differences in the variables measured and the impact of genetics on bird behaviour.

An analysis of the albumen corticosterone concentration data was conducted in the same manner. As the corticosterone data were only available for the Australian egg farms, the analysis was conducted using a bivariate correlation of the Australian data set, followed by a stepwise linear regression analysis.

1.4 Results

The variation in avoidance behaviour observed in different sheds will be presented first, followed by the possible causes of this variation in the following sections. These sections will be presented in the same order as the components of the human-animal interactions model, described in Section 2.2.4 of the literature review. That is, the factors that are associated with stockperson behaviour will be presented, followed by the factors associated with fear of humans in the hens, and finished with the factors associated with productivity.

1.4.1 Between-shed variation in avoidance behaviour to the researcher displayed by laying hens

Avoidance behaviour of the hens to the researcher varied substantially between sheds, and is depicted in Figure 1. The large degree of variation in the avoidance behaviour shown by the hens in different sheds indicates that the factors influencing this avoidance response also vary between sheds. As will be discussed in further detail in the following sections, this variation can be partially explained by the human-animal relationship. Other aspects of the human-animal relationship, such as human attitudes, human behaviour and shed parameters also varied between sheds. The means and ranges of all variables are presented in Table 8, Table 9 and Table 10.

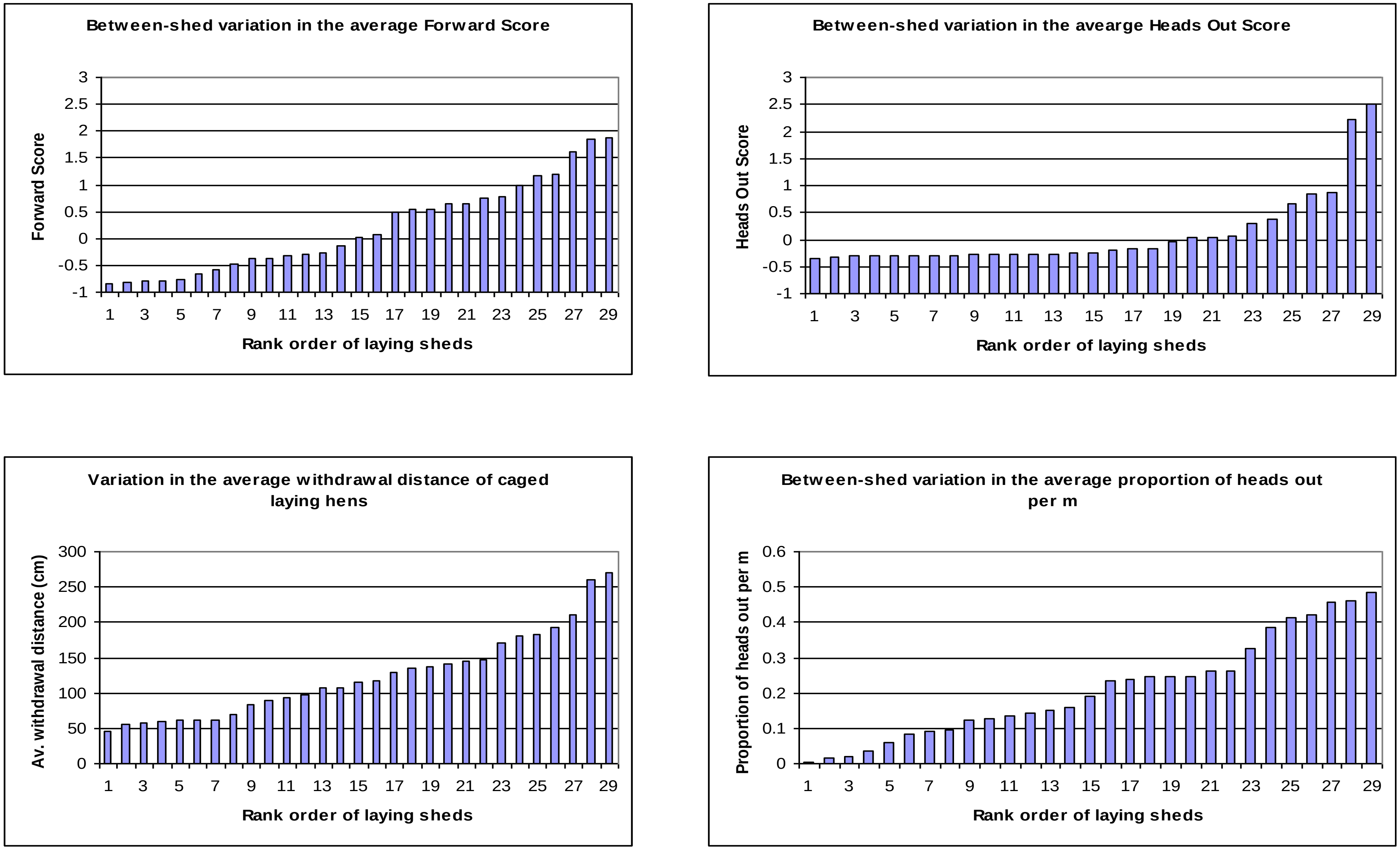


Figure 1 Between-shed variation in the response of commercial laying hens to the experimenter during the behavioural tests

Forward Score = a composite score representing the proportion of birds that were at the cage front during the Approaching Human Test;
Heads Out Score = a composite score representing the proportion of birds that put their heads out of the cage during the Approaching Human Test;
Withdrawal Distance = the minimum distance that the hens would allow the researcher to approach before pulling their heads into the cage during the Stroll Test;
Proportion of heads out per m = the proportion of birds that put their head out of the cage directly in front of the researcher per m of cage length during the Stroll Test

Table 8 **Mean and ranges for the shed parameters, stockperson attitude subscales and empathy, and all stockperson behaviour for all stockpeople over the two-day observation period in the field work. (Back-transformed data are presented in parentheses)**

Variable	Mean	Range
Shed parameters		
Number of stockpeople	3.9	1 – 10
Age of birds (wks)	59.5	26 – 106
No. of birds in shed	66 568.9	1320 – 183 500
Length of aisles (m)	104.2	30.4 – 177.0
Cage width (cm)	52.2	31 - 70
Average no. of birds per cage	5.6	2.9 – 9.0
Average space allowance (cm^2)	473.6	303.5 – 688.8
Lux (log)	0.7 (72.8)	-1.6 – 3.10 (0.0 – 1248.7)
Stockperson attitudes and empathy		
Empathy	15.2	6.0 – 18.0
Sensitivity of stockperson	35.9	25.0 – 43.0
Insensitivity of stockperson	40.8	27.0 – 51.0
Job control	7.8	2.0 – 10.0
Stockperson enjoys job	26.6	12.0 - 35.0
Diligence	34.6	20.0 – 41.0
No job control	15.1	7.0 – 25.0
Unpleasantness of job	31.3	17.0 – 51.0
Negative general attitudes	36.8	25.0 – 55.02
Positive general attitudes	35.4	20.0 – 44.0
Total behaviours for all stockpeople		
Visual / cage (log)	0.3 (1.9)	0.0 – 1.2 (0.0 – 15.9)
Noise / cage (log)	0.2 (0.7)	0.0 – 0.6 (0.0 – 3.1)
Approach/cage (log)	0.2 (0.6)	0.0 – 0.7 (0.0 – 4.1)
Contact / cage (log)	0.3 (1.2)	0.3 – 1.1 (0.1 – 11.6)
Entry / cage	0.1	0.0 – 0.3
Handle / cage	0.0	0.0 – 0.3
Total no. of interactions / cage	0.6 (4.5)	0.1 – 1.5 (0.2 – 31.9)
Near cage / cage	2.0	0.1 – 16.0
Time at start of shed / cage (s)	4.9	0.2 – 14.3
Time in aisle / cage (log) (s)	1.1 (14.7)	0.3 – 1.9 (1.2 – 75.1)
Time in ends of aisles / cage	0.3 (1.8)	0.0 – 0.9 (0.0 – 7.5)
Time stationary / cage (s)	0.0	0.0 – 0.0
Totals secs / cage (s) (log)	1.2 (21.4)	0.4 – 1.9 (1.4 – 86.5)
Av speed of movement (m/s)	0.9	0.2 – 1.8
Max speed of movement (m/s)	2.5	0.4 – 4.9
Min speed of movement (log)	0.1 (0.3)	0.0 – 0.3 (0.0 – 1.0)

Table 9 Mean and ranges for individual stockperson behaviours over the entire two-day observation period, and during bird inspections, collected during the field work. (Back-transformed data are presented in parentheses)

Variable	Mean	Range
Total behaviours for individual stockpeople		
Visual / cage (log)	0.2 (1.3)	0.0 – 1.2 (0.0 – 15.9)
Noise / cage (log)	0.1 (0.3)	0.0 – 0.4 (0.0 – 1.6)
Approach/cage (log)	0.1 (0.5)	0.0 – 0.7 (0.0 – 4.1)
Contact / cage (log)	0.2 (0.8)	0.0 – 1.1 (0.0 – 11.6)
Entry / cage	0.0	0.0 – 0.3
Handle / cage	0.0	0.0 – 0.1
Total no. of interactions / cage (log)	0.4 (3.1)	0.0 – 1.5 (0.0 – 31.9)
Near cage / cage (log)	0.3 (1.4)	0.0 - 1.2 (0.0 - 16.0)
Time at start of shed / cage (log) (s)	0.4 (2.9)	0.0 – 1.1 (0.0 – 10.6)
Time in aisle / cage (log) (s)	0.8 (9.9)	0.2 – 1.9 (0.4 – 75.1)
Time in ends of aisles / cage (log) (s)	0.2 (0.8)	0.0 – 0.8 (0.0 – 4.6)
Time stationary / cage (s)	0.2 (1.0)	0.0 – 0.8 (0.0 – 4.8)
Totals secs / cage (s)	13.6	0.5 – 86.5
Av speed of movement (m/s)	1.0	0.2 – 2.1
Max speed of movement (m/s)	2.1	0.4 – 4.9
Min speed of movement (m/s)	0.4	0.0 – 1.1
Inspection behaviours for individual stockpeople		
Visual / cage (SqRt)	0.3 (0.2)	0.1 – 1.8 (0.0 – 3.2)
Noise / cage (SqRt)	0.0 (0.0)	0.0 – 0.2 (0.0 – 0.1)
Approach/cage (SqRt)	0.3 (0.2)	0.0 – 1.0 (0.0 – 0.9)
Contact / cage (SqRt)	0.3 (0.2)	0.0 – 1.6 (0.0 – 2.5)
Entry / cage (SqRt)	0.1 (0.0)	0.0 - 0.3 (0.0 – 0.1)
Handle / cage (SqRt)	0.0 (0.0)	0.0 – 0.1 (0.0 – 0.0)
Total no. of interactions / cage (SqRt)	0.6 (0.6)	0.1 – 2.6 (0.0 – 6.5)
Near cage / cage (SqRt)	0.5 (0.4)	0.1 – 1.8 (0.0 – 3.4)
Time at start of shed / cage (SqRt) (s)	0.4 (0.2)	0.2 – 1.0 (0.0 – 0.9)
Time in ends of aisles / cage (SqRt) (s)	0.1 (0.0)	0.0 – 0.5 (0.0 – 0.2)
Time in aisle / cage (SqRt) (s)	1.2 (1.8)	0.7 – 3.0 (0.4 – 9.0)
Totals secs / cage (SqRt) (s)	1.3 (2.3)	0.6 – 4.7 (0.3 – 22.1)
Time stationary / cage (SqRt) (s)	0.4 (0.2)	0.0 – 1.1 (0.0 – 1.2)
Av speed of movement (SqRt) (m/s)	0.3 (1.0)	0.1 – 0.5 (0.2 – 2.4)
Max speed of movement (SqRt) (m/s)	0.4 (1.5)	0.1 – 0.8 (0.3 – 4.9)
Min speed of movement (m/s)	0.7	0.0 – 1.2

Table 10 Means and ranges for bird behaviour, albumen corticosterone concentration and productivity variables collected during the field work. (Back-transformed data are presented in parentheses)

Variable	Mean	Range
Bird behaviour		
Forward Score	0.2	-0.8 – 1.9
Heads Out Score (log)	-0.0 (0.1)	-0.2 – 0.5 (-0.3 – 2.5)
Withdrawal distance	123.6	46.1 – 271.1
Proportion of heads out / m	0.2	0.0 – 0.5
Albumen corticosterone concentration (ng/ml)	1.6	1.1 – 2.2
Productivity		
Peak Hen Day Production (PHDP) (%)	95.7	93.1 – 98.9
No. of weeks within 2 % of PHDP	6.8	1.0 – 15.0
No. of weeks within 5 % of PHDP	15.5	1.0 – 27.0
% Mortality at 29 wks (log)	0.2 (0.6)	0.1 – 0.5 (0.1 – 2.3)
Age at PHDP (wks) (log)	1.5 (31.8)	1.4 – 1.7 (23.0 – 47.0)
Egg weight (g)	62.8	54.9 – 66.9

1.4.2 Relationships between stockperson behaviours and stockperson attitudes

The analyses of stockperson attitudes and behaviours are presented in three sections. The first section presents the correlations between each of the ten attitude subscales only. The second section presents the relationships between attitudes and all stockperson behaviours recorded over the two-day observation period for individual stockpeople. This measurement included all behaviours observed for each stockperson, and provides a more encompassing assessment of stockperson behaviour in the laying shed. The third section presents the relationships between attitudes and the stockperson behaviours observed whilst doing a single standard task: inspecting the birds for mortalities. This measurement was included to account for the fact that not all stockpeople were observed doing the same task over the two-day period of observation, and thus their behaviours would differ due to the type of work that they were doing. For the purpose of describing these latter two separate analyses, the total behaviours for each stockperson will be referred to as ‘Total behaviours’ and the behaviours observed during inspections will be referred to as ‘Inspection behaviours’.

1.4.2.1 Partial correlations between the attitude subscales

A partial correlation was conducted with country partialled out to determine any relationships between each of the ten attitude subscales. The results are presented in Table 11. Positive attitudes and empathy correlated positively with each other, and negatively with negative attitudes. Likewise, negative attitudes correlated positively with each other, and negatively with the positive attitudes. All attitudes were well correlated, with the exception of ‘Sensitivity of stockperson’ and ‘Insensitivity of stockperson’. ‘Sensitivity of stockperson’ was not correlated with three of the nine attitude subscales, and ‘Insensitivity of stockperson’ was not significantly correlated with any of the attitude subscales, although there was a tendency ($P < 0.10$) to be correlated with ‘Negative general attitudes’.

1.4.2.2 Partial correlations between the attitude subscales, total behaviours for individual stockpeople and shed parameters

A partial correlation that was conducted with country partialled out determined that six attitude subscales and empathy were significantly correlated with the total stockperson behaviours. The results of this correlation analysis are presented in Table 12. Generally, positive attitudes and empathy were associated with less noise, and negative attitudes were associated with more noise, faster speed of movement, more total time in the shed and less time at the start of the shed or in the ends of the aisles. The minimum speed of movement was greater when stockpeople agreed with statements about being insensitive to the behaviour of the hens ($P < 0.05$), and the average speed of movement tended to be greater when stockpeople found their job unpleasant ($P < 0.10$). Noise was moderately to strongly correlated with empathy and several attitudes. Stockpeople made less noise when they agreed with statements about empathy ($P < 0.01$), were sensitive to the behaviour and feelings of the birds ($P < 0.01$), enjoyed their job ($P < 0.05$) and had positive general attitudes toward the hens ($P < 0.01$). Stockpeople who had negative general attitudes toward the hens made more noise ($P < 0.01$) and spent more time in the shed ($P < 0.05$), but spent less time in the ends of the aisles ($P < 0.05$). The total amount of time spent in the shed tended to be lower when stockpeople agreed with statements about empathy ($P < 0.10$). Stockpeople who agreed that their job was unpleasant spent less time at the start of the shed ($P < 0.05$) and in the ends of the aisles ($P < 0.05$).

Table 11 Partial correlation coefficients (P < 0.10) between all of the stockperson attitude subscales and empathy, with country partialled out

	Empathy	Sensitivity of SP	Insensitivity of SP	Job Control	SP enjoys job	Diligence	No job control	Unpleasantness of job	Neg Gen Atts	Pos Gen Atts
Empathy	1.00	0.64 **		0.63 **	0.63 **	0.65 **	-0.54 **	-0.58 **	-0.74 **	0.83**
Sensitivity of SP		1.00		0.47 **	0.67 **	0.65 **			-0.51 **	0.60 **
Insensitivity of SP			1.00						0.34 †	
Job Control				1.00	0.56 **	0.41 *	-0.63 **	-0.56 **	-0.31 †	0.51 **
SP enjoys job					1.00	0.56 **	-0.37 *	-0.54 **	-0.39 *	0.66 **
Diligence						1.00	-0.42 *	-0.45 *	-0.45 *	0.58 **
No job control							1.00	0.75 **	0.51 **	-0.52 **
Unpleasantness of job								1.00	0.49 **	-0.60 **
Neg Gen Atts									1.00	-0.85 **
Pos Gen Atts										1.00

† P < 0.10 * P < 0.05 ** P < 0.01, a blank space indicates that P > 0.10, df = 28

SP is an abbreviation for stockperson; **Neg Gen Atts** is an abbreviation for Negative General Attitudes; **Pos Gen Atts** is an abbreviation for Positive General Attitudes

Table 12 Partial correlation coefficients (P < 0.10) between stockperson attitude subscales and empathy, the total behaviours for individual stockpeople, and shed parameters, with country partialled out

	Empathy	Sensitivity of SP	Insensitivity of SP	Job Control	SP enjoys job	Diligence	No job control	Unpleasantness of job	Neg Gen Atts	Pos Gen Atts
Av SOM								0.35 †		
Min SOM			0.38 *							
Noise / cage (log)	-0.63 **	-0.51 **			-0.42 *				0.58 **	-0.58 **
Total Secs/cage	-0.33 †								0.44 *	
Time at SOS / cage (log)								-0.39 *		
Time in Ends of Aisles/cage (log)								-0.38 *	0.37 *	
No. of stockpeople	-0.50 **	-0.32 †		-0.33 †			0.31 †		0.45 *	-0.37 *

† P < 0.10 * P < 0.05 ** P < 0.01, a blank space indicates P > 0.10, df = 28

SP is an abbreviation of stockperson**; Neg Gen Atts** is an abbreviation of Negative general attitudes; **Pos Gen Atts** is an abbreviation of Positive general attitudes
Av SOM = average speed of movement in all aisles; **Min SOM** = minimum speed of movement observed; **Noise** = all noise made in the shed; **Total Secs** = the total amount of time spent in the shed by an individual stockperson; **Time SOS** = the total amount of time spent at the start or end of the shed by an individual stockperson; **Time in Ends of Aisles** = the total amount of time spent in the ends of the aisles by an individual stockperson, without moving all the way along the aisle; **No of stockpeople** = the total number of stockpeople observed in each laying shed.

The number of stockpeople that were observed in each shed was the only shed parameter that correlated significantly with stockperson attitudes. In sheds with more people working in them, stockpeople agreed less with statements about empathy ($P < 0.01$) and positive general statements about the hens ($P < 0.05$), and agreed more with negative general statements about hens ($P < 0.05$). Stockpeople in these sheds also tended ($P < 0.10$) to agree less with statements about having control over their job and being sensitive to the behaviour of the birds.

To determine whether features of the laying shed were correlated with the total behaviours for individual stockpeople, a partial correlation analysis was conducted with country partialled out. The results are presented in Table 13. Stockpeople working in large sheds, as measured by aisle length, made less cage contacts ($P < 0.01$) and less behaviours near the cages ($P < 0.05$), but spent more time in the shed per cage ($P < 0.05$). They also tended to make less visual-only behaviours ($P < 0.10$) and spend more time in the ends of the aisles ($P < 0.10$). A greater number of stockpeople observed working in the shed was associated with a higher incidence of noise ($P < 0.05$), less cage contacts ($P < 0.05$) and more time spent in the shed ($P < 0.05$). The stockpeople also made more cage entries when the birds were older ($P < 0.05$). Stockpeople working is sheds with a wider cages made more noise ($P < 0.05$), and spent more time in the shed ($P < 0.01$), more time in the ends of the aisles ($P < 0.01$), more time at the start of the shed ($P < 0.01$), and more time standing stationary in the aisles ($P < 0.05$). They also tended to make more cage entries ($P < 0.10$), handle the birds more often ($P < 0.10$), and spend more time in the aisles ($P < 0.10$). Similarly, stockpeople working in sheds with greater space allowances for the hens spent more time in the shed ($P < 0.05$), more time in the start of the shed ($P < 0.05$), and tended to make more noise ($P < 0.10$). Finally, low light levels were associated with less time spent in the shed ($P < 0.01$) and less time in the ends of the aisles ($P < 0.01$), and tended to be associated with less noise ($P < 0.10$), less handling of the birds ($P < 0.10$) and less time spent at the start of the shed ($P < 0.10$).

Table 13 Partial correlation coefficients (P < 0.10) between the total behaviours for individual stockpeople and shed parameters, with country partialled out

	Aisle length	No. of stockpeople	Age of birds (wks)	Cage width (cm)	Space allowance	Lux (log)
Visual/cage (log)	-0.31 †					
Noise/cage (log)		0.44 *		0.39 *	0.35 †	-0.36 †
Contact/cage (log)	-0.49 **	-0.43 *				
Entry/cage			0.44 *	0.32 †		
Handle/cage				0.32 †		-0.31 †
Total secs/cage	0.44 *	0.43 *		0.51 **	0.43 *	-0.50 **
Near cage/cage (log)	-0.41 *					
Time at start of shed/cage (log)				0.46 **	0.37 *	-0.35 †
Time in aisle/cage (log)				0.34 †		
Time ends of aisles/cage (log)	0.32 †			0.51 **		-0.60 **
Time stationary/cage (log)				0.41 *		

† P < 0.10 * P < 0.05 ** P < 0.01, a blank space indicates P > 0.10, df = 28

Visual = behaviours in the aisle that did not approach the cages; **Noise** = all noise made by an individual stockperson; **Contact** = any contact with the cage, feed trough or egg belt; **Entry** = hands or object placed inside cage; **Handle** = toughing or restraining live birds; **Total Secs** = the total amount of time spent in the shed by an individual stockperson over the two-day observation period; **Near cage** = all behaviours performed near the cage, calculated by summing Approach, Contact, Entry and Handle; **Time at Start of Shed** = the total amount of time that an individual stockperson spent at the start or end of the shed; **Time in aisle** = total time in all aisles; **Time in Ends of Aisles** = the total amount of time that an individual stockperson spent in the ends of the aisles, without moving all the way along the aisle; **Time Stationary** = total time spent stationary in the aisles for individual stockpeople; **Aisle length** = length of the aisles in the laying shed (m); **No. of stockpeople** = the total number of stockpeople observed in each laying shed; **Age of birds** = the age of the birds in wks when assessed; **Cage width** = width of the cages in cm; **Space allowance** = floor space of cages in cm^2; **Lux** = light intensity in lux.

Linear regression analyses for total stockperson behaviours

A stepwise linear regression was conducted to explore the relationship between the total behaviours for individual stockpeople, their attitudes and shed parameters. A variable was included in the regression analysis if it correlated with a stockperson behaviour at $P \leq 0.10$, with country partialled out. The results of these analyses are presented in Table 14. Few total stockperson behaviours were predicted by attitudes. The average speed of movement was predicted to be greater for stockpeople who agreed that their job was unpleasant ($P < 0.05$), whilst the minimum speed of movement was predicted to be greater for stockpeople in the US ($P < 0.01$), and when stockpeople agreed with statements about being insensitive to the behaviour of the birds ($P < 0.05$). Stockpeople were predicted to spend more time in the ends of the aisles when they agreed with negative general statements about hens ($P < 0.01$), and when they did not find the job unpleasant ($P < 0.01$). Stockpeople were also predicted to spend more time in the ends of the aisles when the shed was located in Australia ($P < 0.01$), and when the light levels were low ($P < 0.01$). The only other stockperson behaviour to be predicted by attitudes was the amount of noise occurring in the shed. The amount of noise made by an individual was greater for stockpeople who lacked empathy ($P < 0.01$), and when the sheds had a greater space allowance allocated to each bird ($P < 0.01$).

All other stockperson behaviours were predicted by shed parameters, not attitudes. In general, stockpeople in modern sheds spent more time in the sheds but interacted less with the birds. The number of all stockperson behaviours per cage decreased as aisle length increased. Stockpeople spent more time in the shed overall, and in certain areas of the shed, when the aisles were longer, when the cages were wider, and when the birds had a greater space allowance. These shed parameters are all indicative of more modern sheds. There were more cage entries with older birds, and less cage contacts in sheds where a large number of stockpeople worked.

In summary, the total behaviours observed for each stockperson during the two-day observation period were associated with both the attitude subscales and shed parameters. However, only speed of movement, noise and the amount of time spent in the ends of the aisles were predicted by the attitude subscales. Shed parameters predicted the majority of stockperson behaviours that were observed.

Table 14 Results for the linear regression analysis of total stockperson behaviours, shed parameters and the attitude subscales

Stockperson behaviour	Variables included in the final model	β	P-value	Adjusted R^2
Av SOM	Unpleasantness of job	0.36	0.05	0.10
Min SOM	Country	0.73	0.00	0.47
	Insensitivity of stockperson	0.30	0.04	
Visual/cage (log)	Aisle length	-0.57	0.00	0.31
Noise/cage (log)	Empathy	-0.55	0.00	0.57
	Space allowance	0.41	0.00	
Contact/cage (log)	Aisle length	-0.61	0.00	0.31
	No. of stockpeople	-0.27	0.10	
Entry/cage	Age of birds	0.43	0.02	0.16
Handle/cage	Cage width	0.35	0.05	0.10
Near cage/cage (log)	Aisle length	-0.62	0.00	0.37
Total secs/cage	Space allowance	0.61	0.00	0.51
	Cage width	0.37	0.02	
	Aisle length	0.26	0.10	
Time at SOS/cage (log)	Space allowance	0.63	0.00	0.57
	Cage width	0.27	0.05	
Time in aisles/cage (log)	Country	-0.55	0.00	0.43
	Cage width	0.28	0.07	
Time ends of aisles/cage (log)	Country	-0.60	0.00	0.78
	Lux	-0.32	0.00	
	Unpleasantness of job	-0.49	0.00	
	Neg general attitudes	0.49	0.00	
Time stationary/cage (log)	Cage width	0.47	0.01	0.19

Visual = the number of behaviours in the aisle that did not approach the cages for individual stockpeople; **Noise** = all noise made by an individual stockperson; **Contact** = the number of contacts with the cage, feed trough or egg belt for individual stockpeople **Entry** = the number of times the hands or an object are placed inside the cage for individual stockpeople; **Handle** = the number of times live birds are touchedor handled by individual stockpeople; **Near cage** = the number of behaviours performed near the cage, calculated by summing Approach, Contact, Entry and Handle for individual stockpeople;**Total Secs** = the total amount of time spent in the shed by an individual stockperson over the two-day observation period; **Time at SOS** = the total amount of time that an individual stockperson spent at the start or end of the shed; **Time in aisle** = total time in all aisles for individual stockpeople; **Time in Ends of Aisles** = the total amount of time that an individual stockperson spent in the ends of the aisles, without moving all the way along the aisle; **Time Stationary** = total time spent stationary in the aisles for individual stockpeople; **Country** = dummy code for Australia (1) and USA (2); **Aisle length** = length of the aisles in the laying shed (m); **Space allowance** = floor space of cages in cm ; **No. of stockpeople** = the total number of stockpeople observed in each laying shed; **Age of birds** = the age of the birds in wks when assessed; **Cage width** = width of the cages in cm; **Lux** = light intensity in lux.

1.4.2.3 Partial correlations between the attitude subscales, stockperson behaviours during bird inspections and shed parameters

The previous section presented the results for the total behaviours observed for each stockperson over a two day period. However, the number and type of behaviours observed for each stockperson would vary with the type of work that the stockperson was conducting, and the type of work that was observed varied from shed to shed. To gain a more accurate assessment of the relationships between stockperson attitudes and behaviours, the stockperson behaviours recorded during a standard task (bird inspections) was correlated with the attitude subscales in a partial correlation, with country partialled out. The results of this correlation are presented in Table 15. Generally, the incidence of noise during inspections was greater for stockpeople who agreed with negative attitudes, whilst the incidence of cage approaches and time spent stationary in front of the hens was greater when stockpeople disagreed with negative attitudes. Speed of movement was slower when stockpeople agreed with statements about empathy and being sensitive to the behaviour of the birds, and was faster when stockpeople agreed with statements about being insensitive to the behaviour of the birds, felt they had no control over their job, and had negative general attitudes toward the hens and their work. The amount of time spent in the aisles tended to be lower when stockpeople agreed with negative general statements about hens.

In addition, the inspection behaviours were correlated with the shed parameters in a partial correlation, with country partialled out. The results of this correlation analysis are presented in Table 16. Stockpeople working in longer aisles made more noise ($P < 0.05$), but spent less time in the shed ($P < 0.01$) during inspections. As the number of stockpeople working in a shed increased, the number of visual interactions ($P < 0.05$) and time spent in the shed ($P < 0.05$) decreased. The number of cage entries and bird handling bouts ($P < 0.05$) during inspections increased with the age of the birds. Minimum speed of movement tended to be greater in sheds with wider cages, and stockpeople tended to make less cage contacts and spend less time in the ends of the aisles in sheds with a greater space allowance per bird ($P < 0.10$). Finally, high light levels were associated with less time spent at the start of the shed ($P < 0.05$), and tended to be associated with fewer cage approaches ($P < 0.10$).

Linear regression analyses for stockperson behaviours during inspection

A stepwise linear regression was conducted to explore the relationships between the inspection behaviours for individual stockpeople, the attitude subscales and features of the laying shed. A variable was included in the regression analysis if it correlated with a stockperson inspection behaviour at $P \leq 0.10$. The results of these analyses are presented in Table 17.

Table 15 Partial correlation coefficients (P ≤ 0.10) between stockperson attitudes, stockperson behaviours during bird inspections and shed parameters, with country partialled out.

	Empathy	Sensitivity of SP	Insensitivity of SP	Job Control	SP enjoys job	Diligence	No job control	Unpleasantness of job	Neg Gen Atts	Pos Gen Atts
Noise/cage (SqRt)							0.32 †	0.44 *		-0.31 †
Approach/cage (SqRt)			-0.34 †					-0.32 †		
Stationary/cage (SqRt)			-0.39 *							
Time in Aisle/cage (SqRt)									-0.35 †	
Av SOM (log)			0.31 †				0.36 †		0.32 †	
Min SOM	-0.31 †	-0.36 *	0.42 *						0.49 **	

† P < 0.10 * P < 0.05 ** P < 0.01, a blank space indicates P > 0.10, df = 28, (SqRt) denotes a square root transformation

SP is an abbreviation of stockperson; **Neg Gen Atts** is an abbreviation of Negative general attitudes; **Pos Gen Atts** is an abbreviation of Positive general attitudes; **SO**M is an abbreviation of speed of movement.
Noise = the amount of noise made by the stockperson during inspections; **Approach** = the number of times the stockperson or an object the stockperson is holding approaches with 20 cm of the cage front during inspections; **Stationary** = total time spent stationary in the aisles during inspections; **Time in aisle** = total time in all aisles during inspections; **Av SOM** = average speed of movement in all aisles during inspections; **Min SOM** = minimum speed of movement observed during inspections.

Table 16 Partial correlation coefficients (P ≤ 0.10) between stockperson inspection behaviours and shed parameters, with country partialled out.

	Aisle length	No. of stockpeople	Age of birds	Cage width	Space allowance	Lux (log)
Visual/cage (SqRt)		-0.40 *				
Noise/cage (SqRt)	0.38 *					
Approach/cage (SqRt)						-0.35 †
Contact/cage (SqRt)					-0.35 †	
Entry/cage (SqRt)			0.43 *			
Handle/cage (SqRt)			0.37 *			
Time at start of shed/cage (SqRt)						-0.43 *
Time in ends of aisles/cage (SqRt)					-0.32 †	
Total secs/cage (SqRt)	-0.51 **	-0.37 *				
Min SOM				0.32 †		

† P < 0.10 * P < 0.05 ** P < 0.01, a blank space indicates P > 0.10, df = 28. (SqRt) denotes a square root transformation.

Visual = the number of behaviours in the aisle that did not approach the cages during inspections; **Noise** = the amount of noise made by the stockperson during inspections; **Approach** = the number of times the stockperson or an object the stockperson is holding approaches with 20 cm of the cage front during inspections; **Contact** = number of contacts with the cage, feed trough or egg belt during inspections; **Entry** = the number of times the hands or an object were placed inside cage during inspections; **Handle** = the number of times the birds were touched or handled during inspections; **Time Start of Shed** = the total amount of time that an individual stockperson spent at the start or end of the shed during inspections; **Time in Ends of Aisles** = the total amount of time that an individual stockperson spent in the ends of the aisles, without moving all the way along the aisle during inspections; **Total Secs** = the total amount of time spent in the shed by an individual stockperson over the two-day observation period during inspections; **Min SOM** = minimum observed speed of movement during inspections; **Aisle length** = length of aisles in the laying shed; **No of stockpeople** = total number of stocpeople observed in the laying shed; **Age of birds** = the age of the birds in wks when assessed; **Cage width** = width of each cage in cm; **Space allowance** = the floor space in each cage in cm^2; **Lux** = the light intensity at the cage front in lux.

Table 17 Results of the linear regression analysis for stockperson inspection behaviours, attitude subscales and shed parameters

Stockperson behaviour	Variables included in the final model	β	P-value	Adjusted R^2
Noise/cage (SqRt)	Unpleasantness of job	0.44	0.01	0.17
Approach/cage (SqRt)	Country	-0.73	0.00	0.55
	Lux (log)	-0.24	0.06	
Entry/cage (SqRt)	Age of birds	0.41	0.02	0.14
Handle/cage (SqRt)	Age of birds	0.38	0.04	0.12
Stationary/cage (SqRt)	Insensitivity of stockperson	-0.39	0.03	0.15
	Country	-0.35	0.06	
Time in aisles/cage (SqRt)	Country	-0.40	0.03	0.17
	Negative general attitudes	-0.33	0.06	
Time at start of shed/cage (SqRt)	Lux (log)	0.41	0.02	0.24
	Country	-0.34	0.04	
Av SOM	Negative general attitudes	0.31	0.09	0.07
Minimum SOM	Negative general attitudes	0.44	0.01	0.17

(SqRt) denotes a square root transformation.
Noise = the amount of noise made by the stockperson during inspections; **Approach** = the number of times the stockperson or an object the stockperson is holding approaches with 20 cm of the cage front during inspections; **Entry** = the number of times the hands or an object were placed inside cage during inspections; **Handle** = the number of times the birds were touched or handled during inspections; **Time stationary** = the total amount of time spent standing stationary in the aisles by an individual stockperson during inspections; **Time in aisles** = the total amount of time spent in the aisles by an individual stockperson during inspections; **Time Start of Shed** = the total amount of time that an individual stockperson spent at the start or end of the shed during inspections; **Av SOM** = the average speed of movement during inspections; **Min SOM** = minimum observed speed of movement during inspections; **Lux** = light intensity at cage front in lux; Age of birds = age of birds in wks when assessed; **Country** = dummy code for Australia (1) and USA (2).

Five inspection behaviours were predicted by the attitude subscales, although the regression models for these relationships only explained a small amount of the variation (Adjusted $R^2 = 0.07 – 0.17$). Stockpeople who found the job unpleasant made more noise during inspection ($P < 0.01$). The amount of time spent stationary during inspections was predicted to be lower when stockpeople agreed with statements about being insensitive to the behaviour of the birds ($P < 0.05$), and when the shed was located in the US ($P < 0.10$). The amount of time spent in the aisles during inspections was greater for stockpeople in Australia ($P < 0.05$), and when stockpeople disagreed with negative general statements about hens ($P < 0.10$). The average speed of movement ($P < 0.10$) and the minimum speed of movement ($P < 0.01$) were predicted to be faster for stockpeople who agreed with negative general statements about hens.

The remaining stockperson behaviours were predicted by shed parameters only. In general, stockpeople in Australia made more interactions and spent more time in the shed during inspections compared to stockpeople in the US. Stockpeople made more cage entries and handled the birds more when the birds were older, and low light levels were associated with more cage approaches. Low light levels were associated with more time spent at the start of the shed.

1.4.3 Relationships between hen avoidance behaviour to the researcher and stockperson behaviour

Partial correlation analysis

Partial correlations were conducted with country partialled out to determine any relationships between stockperson behaviour and the avoidance behaviour displayed by the hens during the behavioural tests. The results of these correlations for each of the four measures of avoidance behaviour are presented in Table 18. Correlation coefficients at $P \leq 0.10$ are presented to display non-significant trends between the variables.

Significant correlations existed between stockperson behaviour and the avoidance behaviour displayed by the hens during the behavioural tests. The incidence of noise in the shed was strongly and negatively correlated with the Forward Score ($P < 0.01$) and the Heads Out Score (log) ($P < 0.01$), with a higher incidence of noise associated with greater avoidance. With the exception of these noise-behaviour correlations the remaining relationships were positive: low avoidance behaviour to humans (fear) was displayed by the hens on farms where stockpeople spent more time standing stationary in the aisle ($P < 0.05$); made more interactions in close proximity to the birds ($P < 0.01$); approached the cages more often ($P < 0.05$) and contacted the cages, egg belts and feed troughs more often ($P < 0.05$).

Table 18 Partial correlation coefficients (P < 0.1) between hen avoidance behaviour and stockperson behaviour, with country partialled out

	Forward Score	Heads Out Score (log)	Withdrawal distance	Prop heads out/m
Max SOM		0.32 †		
Time stationary / cage		0.38 *		
Noise / cage (log)	-0.65 **	-0.59 **	0.34 †	-0.35 †
Near cage / cage	0.34 †	0.48 *		0.34 †
Approach / cage (log)		0.32 †		0.37 *
Contact / cage (log)		0.40 *		

† P < 0.10 * P < 0.05, ** P < 0.01, a blank space indicates P > 0.10, df = 26,

(Log) denotes a log transformation
SOM is an abbreviation of speed of movement.
Forward Score = a composite score representing the proportion of birds that were at the cage front during the Approaching Human Test; **Heads Out Score** = a composite score representing the proportion of birds that put their heads out of the cage during the Approaching Human Test; **Withdrawal Distance** = the minimum distance that the hens would allow the researcher to approach before pulling their heads back into the cage during the Stroll Test; **Proportion of heads out/ m** = the proportion of birds that put their head out of the cage directly in front of the researcher per m during the Stroll Test; **Max SOM** = maximum observed speed of movement for all stockpeople; **Time Stationary** = total time spent stationary in the aisles for all stockpeople; **Noise** = all noise made in the shed for all stockpeople; **Near cage** = all behaviours performed near the cage, calculated by summing Approach, Contact, Entry and Handle for all stockpeople; **Approach** = the number of times the stockperson or an object the stockperson is holding approaches with 20 cm of the cage front for all stockpeople; **Contact** = the number of contacts with the cage, feed trough or egg belt for all stockpeople.

Several shed parameters were also correlated with hen behaviour, and these results are presented in Table 19. There were non-significant trends for larger sheds to be associated with increased avoidance behaviour; longer aisles and a higher number of birds in the shed were associated with increased avoidance behaviour in the Approaching Human Test (Forward Score and Heads Out Score (log), $P < 0.09$). Group size was significantly correlated with avoidance behaviour, with avoidance being greatest on farms with more birds in the cages ($P < 0.02$). Interestingly, a greater space allowance was associated with a lower proportion of birds with their heads out when approached by a novel human ($P < 0.01$).

Table 19 Partial correlation coefficients (P < 0.1) between hen avoidance behaviour and shed parameters, with country partialled out

	Forward Score	Heads Out Score (log)	Withdrawal distance	Prop heads out/m
No. of birds in shed	-0.35 †	-0.36 †		
Length of aisles (m)		-0.35 †		
No. birds/cage	-0.56 **	-0.47 *		
Space allowance (cm^2)			0.36 †	-0.56 **

† P < 0.10 * P < 0.05, ** P < 0.01, a blank space indicates P > 0.10, df = 26

Forward Score = a composite score representing the proportion of birds that were at the cage front during the Approaching Human Test; **Heads Out Score** = a composite score representing the proportion of birds that put their heads out of the cage during the Approaching Human Test; **Withdrawal Distance** = the minimum distance that the hens would allow the researcher to approach before pulling their heads back into the cage during the Stroll Test; **Proportion of heads out/ m** = the proportion of birds that put their head out of the cage directly in front of the researcher per m during the Stroll Test;

Linear regression analysis of hen behaviour, human behaviour and shed parameters

A stepwise linear regression analysis was conducted to explore the nature of the relationship between hen behaviour, stockperson behaviour and shed parameters. A stockperson behaviour or shed parameter was included in the analysis if it correlated with a bird behaviour at P ≤ 0.10. Country and bird strain were dummy-coded and included in the analysis. The results of the linear regression analysis are presented in Table 20.

Bird strain, country and stockperson behaviour were important predictors of the avoidance behaviour of birds to the researcher, and accounted for between 42% and 74% of the observed variation in avoidance behaviour (Adjusted R^2 = 0.42 – 0.74). Country and bird strain contributed significantly to the prediction of bird behaviour, with hens in Australia and brown strains of hen predicted to show less avoidance behaviour. The amount of noise made in the laying shed was included in all four models, and was positively associated with avoidance behaviour. That is, as the incidence of noise in the shed increased, fewer birds were predicted to be present at the cage front during the behavioural tests. Other stockperson behaviours that predicted avoidance behaviour in the hens were the number of behaviours that stockpeople made in close proximity to the cages (Near cage/cage), the amount of time spent stationary in the aisles (Stationary/cage), the number of cage approaches made (Approach/cage (log), and the maximum speed of movement (Max SOM). The regression models predicted that more birds would be present at the cage front during the behavioural tests (indicating low avoidance of humans) in sheds in which stockpeople displayed more of these types of behaviours.

Table 20 Results of the linear regression analysis for hen avoidance behaviour, stockperson behaviour and shed parameters

Hen behaviour	Variables included in the final model	β	P-value	Adjusted R^2
Forward Score	Strain	-0.67	0.00	0.71
	No. birds/cage	-0.24	0.07	
	Noise/cage (log)	-0.24	0.08	
	Near cage/cage	0.21	0.06	
Heads Out Score (log)	Stationary/cage	0.51	0.00	0.74
	Noise/cage (log)	-0.43	0.00	
	No. birds/cage	-0.30	0.02	
	Max SOM	0.26	0.02	
	Strain	-0.26	0.07	
Withdrawal Distance	Country	0.77	0.00	0.42
	Noise/cage (log)	0.30	0.08	
Proportion Heads Out/ m	Approach/cage (log)	0.54	0.00	0.46
	Strain	-0.38	0.03	
	Noise/cage (log)	-0.34	0.04	

df = 28

Forward Score = a composite score representing the proportion of birds that were at the cage front during the Approaching Human Test; **Heads Out Score** = a composite score representing the proportion of birds that put their heads out of the cage during the Approaching Human Test; **Withdrawal Distance** = the minimum distance that the hens would allow the researcher to approach before pulling their heads back into the cage during the Stroll Test; **Proportion of heads out/ m** = the proportion of birds that put their head out of the cage directly in front of the researcher per m during the Stroll Test**;** **Strain** = dummy code for brown birds (1) or white birds (2); **Noise** = all noise made in the shed for all stockpeople; **Near cage** = all behaviours performed near the cage, calculated by summing Approach, Contact, Entry and Handle for all stockpeople; **Time Stationary** = total time spent stationary in the aisles for all stockpeople; **Approach** = the number of times the stockperson or an object the stockperson is holding approaches with 20 cm of the cage front for all stockpeople; **Max SOM** = maximum observed speed of movement for all stockpeople; **Country** = dummy code for Australia (1) and USA (2); **Approach** = the number of times the stockperson or an object the stockperson is holding approaches with 20 cm of the cage front for all stockpeople;

Partial correlations between hen behaviour and stockperson attitudes

A partial correlation analysis was conducted with country partialled out to determine relationships between the avoidance behaviour of the hens and the attitudes of the stockpeople. A correlation coefficient was presented if it correlated with a measure of hen behaviour to a significance of $P \leq 0.10$. The results of this analysis are presented in Table 21.

Table 21 Partial correlation coefficients ($P \leq 0.10$) between hen avoidance behaviour and stockperson attitudes, with country partialled out

	Forward Score	Heads Out Score (log)	Withdrawal distance	Prop Heads Out / m
Empathy	0.54 **	0.45 *		
Sensitivity of SP	0.53 **	0.31 †		
Insensitivity of SP		-0.32 †		
SP enjoys job	0.34 †			
Neg Gen Attitudes	-0.55 **	-0.59 **		-0.40 *
Pos Gen Attitudes	0.49 **	0.37 *		

† P < 0.10 * P < 0.05, ** P < 0.01, a blank space indicates P > 0.10, df = 28

SP is an abbreviation of stockperson

Forward Score = a composite score representing the proportion of birds that were at the cage front during the Approaching Human Test; **Heads Out Score** = a composite score representing the proportion of birds that put their heads out of the cage during the Approaching Human Test; **Withdrawal Distance** = the minimum distance that the hens would allow the researcher to approach before pulling their heads back into the cage during the Stroll Test; **Proportion of heads out/ m** = the proportion of birds that put their head out of the cage directly in front of the researcher per m during the Stroll Test**;**

Many attitude subscales were significantly correlated with the 'Forward Score' and the 'Heads Out Score (log)', whilst only one attitude subscale was correlated with the results of the Stroll Test. Empathy and positive attitudes, such as 'Sensitivity of stockperson', 'Stockperson enjoys job' and 'Positive General Attitudes', were correlated with more birds present at the cage front during the behavioural tests, while attitudes that can be classified as negative, such as 'Insensitivity of stockperson', were correlated with fewer birds present at the cage front.

A linear regression analysis to predict hen avoidance behaviour using the attitude subscales, stockperson behaviours and shed parameters that were correlated with bird behaviour was not deemed appropriate due to the different scales of measurement used. The stockperson behaviours were measured using shed averages, whilst the stockperson attitudes were measured for individual stockpeople.

1.4.4 Albumen corticosterone concentration

Bivariate correlation analysis

The corticosterone concentration of egg albumen was only available for Australian farms. This removed the issue of country differences in the data, but also meant that the sample size was reduced (n = 20). A bivariate correlation was conducted to determine the existence of relationships between albumen corticosterone concentration, human behaviour, hen behaviour and shed parameters. The results of this correlation are presented in Table 22.

Table 22 Bivariate correlation coefficients ($P \leq 0.1$) for albumen corticosterone concentration, hen avoidance behaviour, stockperson behaviour and shed parameters

Type of variable	Variables	r	P-value
Hen behaviour	Withdrawal Distance	-0.64	0.00
	Prop. Heads Out/m	0.50	0.03
Shed parameters	Space Allowance	-0.45	0.05
Stockperson behaviour	Noise/cage (log)	-0.54	0.02

df = 18

Withdrawal Distance = the minimum distance that the hens would allow the researcher to approach before pulling their heads back into the cage during the Stroll Test; **Proportion of heads out/ m** = the proportion of birds that put their head out of the cage directly in front of the researcher per m during the Stroll Test; **Space allowance** = floor space of cage in cm^2; **Noise** = the total amount of noise made by all stockpeople.

Albumen corticosterone concentration was significantly correlated with two aspects of hen behaviour. It was negatively correlated with the Withdrawal Distance ($P < 0.01$) and positively correlated with the Prop. Heads Out/m ($P < 0.05$), indicating that farms with a higher albumen corticosterone concentration also displayed less avoidance behaviour during the Stroll Test. In addition, farms with greater space allowance had lower albumen corticosterone concentration ($P < 0.05$).

Only one stockperson behaviour was significantly correlated with corticosterone concentration. A high incidence of noise in the shed was associated with less corticosterone in the egg albumen ($P < 0.05$). There were no relationships between egg albumen corticosterone concentration and the attitude subscales or production variables.

Linear regression analysis of albumen corticosterone concentration on Australian farms

A stepwise linear regression analysis of the Australian field data was conducted to determine the nature of the relationship between albumen corticosterone concentration, hen behaviour, stockperson behaviour and shed parameters. A variable was included in the analysis if it correlated with albumen corticosterone concentration at $P \leq 0.10$.

The Withdrawal Distance was the only variable that remained in the final model ($\beta = -0.64$, $P = 0.00$). This model explained 37% of the variation observed in albumen corticosterone concentration (Adj $R^2 = 0.37$), and predicted that albumen corticosterone concentration would be lower in sheds where the birds displayed high fear of humans, based on their response to the researcher in the Stroll Test.

1.4.5 Factors associated with hen productivity

Partial correlation analysis

A partial correlation was conducted with country partialled out to determine any relationships between hen behaviour, shed parameters, stockperson behaviour and productivity variables. The results are presented in Table 23. Both the Forward Score and the Heads Out Score (log) were significantly and negatively correlated with all three measures of egg production ($P < 0.05$). The number of weeks that hens stayed within 2% of their peak rate of lay was also negatively correlated with avoidance behaviour in the Stroll Test ($P < 0.05$). That is, the farms that had the highest egg production were also the farms where the birds displayed the greatest fear of humans. Peak hen day production was also positively correlated with the number of birds per cage ($P < 0.05$), with production being higher in sheds with large group sizes. There were tendencies for peak production (PHDP) to be greater when the average speed of movement by the stockpeople was slower ($P < 0.10$), and when there was a higher incidence of noise in the shed ($P < 0.10$).

The birds also remained within 2% of their peak rate of lay for longer in sheds with low light levels ($P < 0.05$) and when there was a high incidence of noise in the shed ($P < 0.05$). The birds tended ($P < 0.10$) to sustain a high rate of lay (PHDP2% and PHDP5%) when there was a greater space allowance, when the flock was older, and when the stockpeople spent more time in the ends of the aisles.

There was a trend for high mortality to be associated with lower fear in relation to the Withdrawal Distance ($P < 0.10$), and more time spent in the ends of the aisles by the stockpeople ($P < 0.10$). The age at peak egg production was greater in sheds with longer aisles ($P < 0.05$), and tended to be greater in sheds with larger flocks ($P < 0.10$). Age at peak production was positively correlated with the amount of time spent in the ends of the aisles by the stockpeople ($P < 0.01$), and tended to be correlated with faster speed of movement ($P <0.10$). Egg weight was lower in sheds where stockpeople made more cage entries ($P < 0.05$), and tended to be lower when there were more interactions close to the cage ($P < 0.10$) and more cage contacts ($P < 0.10$).

Table 23 Partial correlation coefficients (P < 0.1) between productivity variables, hen avoidance behaviour, shed parameters and stockperson behaviour, with country partialled out.

	PHDP	PHDP 2%	PHDP 5%	Age at PHDP (log)	Mortality (log)	Egg Wt
Forward Score	-0.46 *	-0.66 **	-0.51 *			
Heads Out Score (log)	-0.43 *	-0.44 *	-0.37 †			
Withdrawal Distance		0.42 *			-0.35 †	
Prop Heads Out/m		-0.47 *				
Age of birds (wks)			0.37 †			
Space Allowance (cm2)		0.40 †				
Aisle length (m)				0.43 *		
Lux (log)		-0.48 *				
No. birds/cage	0.41 *					
No. birds in shed				0.36 †		
Av SOM	-0.38 †					
Max SOM				0.37 †		
Time in ends of Aisles / cage (log)		0.39 †		0.57 **	0.37 †	
Near Cage / cage						-0.38 †
Noise / cage (log)	0.41 †	0.46 *	0.37 †			
Contacts / cage (log)						-0.36 †
Entry / cage						-0.49 *

† P < 0.10 * P < 0.05 ** P < 0.01, a blank space indicates P > 0.10, df = 21 (df for egg weight = 22)

PHDP is an abbreviation of Peak Hen Day Production; SOM is an abbreviation of speed of movement
Forward Score = a composite score representing the proportion of birds that were at the cage front during the Approaching Human Test; **Heads Out Score** = a composite score representing the proportion of birds that put their heads out of the cage during the Approaching Human Test; **Withdrawal Distance** = the minimum distance that the hens would allow the researcher to approach before pulling their heads back into the cage during the Stroll Test; **Proportion of heads out/ m** = the proportion of birds that put their head out of the cage directly in front of the researcher per m during the Stroll Test**;** **Age of birds** = age at which the birds were assessed; **Space allowance** = floor space of cage in cm^2**; Aisle length** = length of aisles in laying shed; **Lux** = light intensity at cage front in lux; **No. birds in cage** = average no. of birds in each focal cage; **No. birds in shed** = size of the flock in each focal shed; **Av SOM** = average speed of movement of all stockpeople; **Max SOM** = maximum speed of movement observed for all stockpeople; **Time in ends of aisles** = total amount of time spent in the ends of the aisles, without walking all the way along, for all stockeople; **Near Cage** = the total number of stockperson behaviours that occurred near to the birds for all stockpeople; **Noise** = the total amount of noise made by all stockpeople; **Contacts** = the total number of contacts with the cage, feed trough and egg belt for all stockpeople; **Entry** = the total number of cage entries made by all stockpeople; **PHDP** = peak hen day production of eggs; **PHDP 2%** = the no. of weeks that the flock laid at a rate of within 2% of their peak egg production; **PHDP 5%** = the no. of weeks that the flock laid at a rate of within 5% of their peak egg production; **Age at PHDP** = age in wks at which the birds reached peak production; **Mortality** = cumulative mortality at 29 wks of age; **Egg wt** = average egg weight on the day of behavioural testing.

Linear regression analysis of productivity, hen behaviour, human behaviour and shed parameters

A stepwise linear regression analysis was conducted for each productivity variable to determine the nature of the relationship between production, hen avoidance behaviour, stockperson behaviour and shed parameters. A variable was included in the regression analysis if it correlated with the production variable to a significance of $P \leq 0.10$. Country and bird strain were dummy-coded and included in the analyses. The results of the linear regression analysis are presented in Table 24.

Hen productivity was predicted by a combination of bird behaviour, stockperson behaviour and shed parameters. Peak egg production was predicted to be greater in sheds where the stockpeople made more noise ($P < 0.05$) and moved more slowly ($P < 0.05$). The number of weeks that the birds sustained egg production within 2% of their peak was predicted to be higher when the light levels were lower ($P < 0.05$), when fewer birds put their heads out of the cage during the Stroll Test ($P < 0.05$), and when stockpeople made more noise in the shed ($P < 0.10$). The number of weeks that the birds sustained egg production within 5% of their peak was predicted to be greater when there was more noise occurring in the shed ($P < 0.05$).

The age at peak egg production was predicted to be greater when the stockpeople used a faster maximum speed ($P < 0.05$), and mortality at 29 wks of age was predicted to be greater when stockpeople spent more time in the ends of the aisles ($P < 0.05$). Average egg weight was predicted to be higher on Australian farms ($P < 0.01$) and when stockpeople made less cage entries ($P < 0.01$).

Table 24 Results of the linear regression analysis for hen productivity, hen avoidance behaviour, stockperson behaviour and shed parameters

Production variable	Variables included in the final model	β	P-value	Adjusted R^2
PHDP	Noise/cage (log)	0.57	0.02	0.39
	Av SOM	-0.34	0.05	
PHDP 2%	Lux (log)	-0.40	0.03	0.37
	Prop heads out/m	-0.41	0.02	
	Noise/cage (log)	0.30	0.10	
PHDP 5%	Noise/cage (log)	0.44	0.03	0.16
Age at PHDP (log)	Max SOM	0.46	0.02	0.17
Mortality (log)	Time in Ends Aisles/cage (log)	0.41	0.04	0.13
Egg Wt	Country	-0.56	0.00	0.44
	Entry / cage	-0.42	0.01	

df = 23 (df = 27 for Av Egg Wt)

PHDP = peak hen day production of eggs; **PHDP 2%** = the no. of weeks that the flock laid at a rate of within 2% of their peak egg production; **PHDP 5%** = the no. of weeks that the flock laid at a rate of within 5% of their peak egg production; **Age at PHDP** = age in wks at which the birds reached peak production; **Mortality** = cumulative mortality at 29 wks of age; **Egg wt** = average egg weight on the day of behavioural testing; **Noise** = the total amount of noise made by all stockpeople; **Contacts** = the total number of contacts with the cage, feed trough and egg belt for all stockpeople; **Av SOM** = average speed of movement of all stockpeople; **Lux** = the light intensity at the cage front in lux; **Prop heads out/ m** = the proportion of birds that put their head out of the cage directly in front of the researcher per m during the Stroll Test**;Max SOM** = maximum speed of movement observed for all stockpeople; **Time in ends of aisles** = total amount of time spent in the ends of the aisles, without walking all the way along, for all stockeople; **Country =** dummy code for Australia (1) and USA (2); **Entry** = the total number of cage entries made by all stockpeople;

1.4.6 Summary of Results

Large differences in shed parameters, bird behaviour and stockperson behaviour were found between Australian and US egg farms. Australian sheds were smaller and less densely stocked, and used brown strains of laying hen rather than white. Australian hens also demonstrated markedly less fear of humans than the birds in the US. Stockpeople in Australia spent more time in the shed per cage, and interacted with their hens more often than their American counterparts.

Stockperson attitudes and empathy were related to stockperson behaviour. In general, negative attitudes were associated with faster speed of movement, more noise, less time spent in the aisles and less time standing stationary in the aisles. The avoidance behaviour of the hens was predicted to be greater when stockpeople made more noise in the shed, and lower when stockpeople spent more time stationary in the aisle, moved faster and made more behaviours in close proximity to the birds. Increased avoidance behaviour was associated with lower concentrations of corticosterone in egg albumen, and increased egg production.

1.5 Discussion

The results presented in this chapter describe the significant relationships that exist between stockperson attitudes and stockperson behaviour, stockperson behaviour and hen behaviour, and hen behaviour, egg albumen corticosterone concentration and productivity. It should be noted that these relationships do not demonstrate causality, merely relationships that have been observed. As this research was conducted in the field it was impossible to account for every factor that may impact on the behavioural variables being measured. Causality can only be determined through the manipulation of relevant variables in a highly controlled experimental setting, and this research will be presented in the following chapters. Meanwhile, the relationships that were observed in the field and their possible causes are discussed in the following sections.

1.5.1 Factors associated with the behaviour of stockpeople in laying sheds

The attitude subscales were well correlated with each other, with the exception of the attitude subscale ‘Insensitivity of stockperson’. These correlations indicate the presence of an attitude system, in which a series of related attitudes are consistent (Hemsworth and Coleman, 1998). Consistent responses to behavioural belief questions reflect the stockpersons underlying attitude toward particular kinds of interactions with animals (Hemsworth and Coleman, 1998). This result is consistent with the cognitive-dissonance theory, in which a person will experience dissonance if two attitudes do not evaluate a behaviour in the same manner and will take action to modify the dissonant behaviour or attitude so that they achieve consonance (Festinger, 1957, Fishbein and Ajzen, 1975).

The general attitudes were also well correlated with the other behavioural attitudes and empathy. Negative general attitudes were positively correlated with the other negative attitude subscales, and positive general attitudes were positively correlated with the other positive attitude subscales. This suggests that the underlying beliefs that stockpeople have about the hens are indirectly influencing their behavioural attitudes by determining the state of their general attitudes, and is consistent with Ajzen and Fishbein’s Theory of Reasoned Action (Fishbein and Ajzen, 1975, Waiblinger et al., 2002). However, unlike the predictions of the Theory of Reasoned Action, stockperson behaviour was also predicted by general attitudes. For example, negative general attitudes predicted speed of movement during bird inspections, with more negative beliefs associated with faster speed. Potentially, a stockperson who is moving faster would spend less time observing and interacting with the hens, resulting in less opportunity to recognise hen behaviour and thus less opportunity to reappraise the negative beliefs that the stockperson holds about the hens.

Several stockperson behaviours were associated with the attitude subscales, empathy and the features of the laying shed. The attitude subscales predicted similar stockperson behaviours both in terms of the total observations made for each individual over the two day observation period, and also during the standard task of bird inspections. These predictions were all in the expected directions.

Stockpeople made more noise when they agreed with negative attitudes, such as ‘Negative general attitudes’, ‘No job control’ and ‘Unpleasantness of job’, and made less noise when they agreed with empathy and positive attitudes, such as ‘Sensitivity of stockperson’, ‘Stockperson enjoys job’ and ‘Positive general attitudes’. It would appear that stockpeople who were aware of the impact of their behaviour on the birds and enjoyed their work did not make as much noise as stockpeople who found the job unpleasant. It should be noted that the cause-effect relationship is unknown, and a high amount of noise could have caused stockpeople to dislike their job. The majority of noise in the shed was made during cleaning, which in itself is disliked by stockpeople, and it is plausible to suggest that stockpeople who must conduct a lot of cleaning may dislike their job more. This is supported by tendency for noise to be correlated with the attitude ‘No job control’, as having no perceived control over performing an unpleasant job is likely to cause the stockperson to dislike the job even more.

The total amount of noise made by individual stockpeople was predicted by their empathy toward the birds and the space allowance of the cages. Whilst it is understandable that stockpeople who are empathetic toward the birds would make less noise due to the disturbance it may cause to the birds, the relationship between the noise and space allowance was unexpected. This relationship may be simply a matter of sheds with a certain cage-size requiring more noise to be made during husbandry. For example, the more modern sheds had larger cages and were more often cleaned with motorised equipment than the older sheds with smaller cages. The relationship may also be due to the space allowance influencing the appearance of the birds, such as improving their plumage condition, which may encourage an empathetic view of the hens. Empathy was a strong predictor of the amount of noise made in the shed, and any factor that increases empathy may result in less noise made in the shed.

The amount of noise made during inspections was predicted by how unpleasant the stockperson found the work. As there was no requirement for the stockpeople to make noise during inspections, in comparison to tasks such as cleaning, then any noise made by the stockperson is entirely under their control and thus more likely to be determined by their attitudes. Stockpeople who found their work unpleasant may have made more noise during inspections because they cared little about their actions, and were careless when handling equipment.

In terms of speed of movement, stockpeople moved faster when they agreed with negative attitudes, such as ‘Insensitivity of stockperson’, ‘No job control’, ‘Unpleasantness of job’ and ‘Negative general attitudes’, and moved more slowly when they agreed with statements about empathy and the ‘Sensitivity of stockperson’. Speed of movement during inspections was only predicted by negative general attitudes, whilst overall speed of movement during the two day observation period was predicted by how unpleasant the stockperson found the job and their insensitivity to the behaviour of the birds. Thus it would appear that stockpeople who move more slowly are more aware of their impact on the birds Stockpeople who move quickly are more inconsistent with previous research, in which the speed of movement of stockpeople on dairy farms (Breuer et al., 2000) and broiler farms (Cransberg et al., 2000) was not correlated with attitudes. However, these studies generally measured different attitudes to those used in the present study. Breuer et al. (2000) used a composite score of attitudes that were significantly correlated with the frequency of negative tactile interactions with dairy cows. These attitudes were unlikely to be related to the speed at which the stockperson moved the cows into the dairy for milking, as they did not specifically address the stockperson’s attitude toward their speed of movement. The study by Cransberg et al. (2000) measured five different attitudes, but only one was in common with the present study. This attitude related to the stockperson’s enjoyment of the job, and was not associated with the stockperson’s speed of movement in the broiler shed. It is possible that the speed of movement for stockpeople in the study by Cranberg et al. (2000) was influenced by their attitudes, however the attitudes relating to this behaviour were not assessed.

Several measures of the amount of time spent in the shed were associated with negative attitudes. Stockpeople with negative general attitudes spent less time in the aisles during inspections, and stockpeople who found their work unpleasant spent less time at the start of the shed and in the ends of the aisles. Work conducted at the start of the shed and in the ends of the aisles was usually related to maintenance and cleaning and it would appear that stockpeople who disliked the hens and their job were avoiding or rushing through tasks that they did not like. However, the overall amount of time spent in the shed and the amount of time spent in the ends of the aisles were positively correlated with negative general attitudes. It is possible that these relationships were a result of more time spent in the shed causing the stockperson's general attitudes towards hens to become more negative.

Relationships between attitudes and the types of behaviours displayed by the stockpeople were only found for the observations made during the standard task of inspections. Both the number of cage approaches and the time spent stationary were negatively correlated with the attitude 'Insensitivity of stockperson', although only the amount of time spent stationary was predicted by this attitude in the regression analysis. In addition, stockpeople who agreed that their job was unpleasant made fewer cage approaches. These relationships may indicate the degree of volitional control that stockpeople have over these specific behaviours. The amount of time spent stationary may have been related to the amount of time that the stockperson was willing to commit to performing certain tasks during inspections, such as removing dead birds from a cage or inspecting the feed troughs and drinkers. The amount of time spent performing these behaviours was decided by the stockperson, and stockpeople who were unaware of the effect of their presence on the birds may also have been less dedicated to performing these tasks. In relation to the cage approaches, these behaviours by definition did not involve any tactile contact with the cages or the birds, and did not serve any other purpose during inspections other than to provide information to the stockperson. During inspections they were generally performed to check the status of the feed troughs or the condition of the birds, and stockpeople who dislike their work may have been less motivated to perform these behaviours unless necessary.

Interestingly, the number of stockpeople working in the shed was correlated with the attitudes of these stockpeople. A larger number of stockpeople working in the shed was correlated with more negative attitudes and less positive attitudes. Conformity theory states that an individual's attitudes will shift toward the norm (Bernheim, 1994), and it appears to be the case in this study. Sheds with many stockpeople working in them were generally located on larger farms with more staff, and the stockpeople in these sheds tended to believe that they had less job control, had poorer general attitudes and were less aware of the sensitivity and behaviour of the laying hens. A study by Lensink (2000) reported a somewhat similar effect, in which the size of the farm was related to how important the stockpeople considered general contact with veal calves to be for the success of the farm. However, this effect was in the opposite direction to that of the present study, with stockpeople on larger farms considering contact with veal calves to be more important, indicating an improvement in attitudes as the size of the farm increased. A possible explanation for the relationship between farm size and the increase in negative stockperson attitudes may relate to the independence of the stockpeople to conduct their work in their own manner. Large farms in the pig industry have been described as causing a reduction in the heterogeneity of attitudes and behaviour compared to small independent farms (Coleman et al., 1998). This reduction was attributed to the presence of other stockpeople whilst working, which reduced the opportunity of stockpeople to behave totally independently. The presence of additional stockpeople in the present study may been responsible for the shift toward negative attitudes.

In summary, negative stockperson attitudes were associated with more noise, faster speed of movement, more time in the shed, less time spent stationary, less time at the start of the shed and less time in the ends of the aisles. In retrospect, assessing more specific behavioural attitudes may have resulted in stronger attitude-behaviour relationships. It would have been beneficial to assess the attitudes of stockpeople toward those behaviours that were key determinants of avoidance behaviour in the hens, such as the attitudes toward making noise in the shed or interacting within close proximity to the hens. Stockperson attitudes were also correlated with the number of people working in their shed, with poorer attitudes associated with more stockpeople. It may have also proved beneficial to have assessed stockperson attitudes in relation to the subjective norms that occur on egg farms.

1.5.2 Factors associated with the behaviour of commercial laying hens

Reduced avoidance of the researcher during behavioural testing was associated with the stockperson approaching and remaining near the laying hens more often. In addition, the behaviour of the laying hens was significantly related to the strain of hen, their country of origin, and features of the laying shed. It should be noted that no comparison was made between the behaviour of the birds when approached by the researcher during the behavioural tests, and the behaviour of the birds when approached by the stockperson. The behaviour observed during the testing situation was the response of the birds to a novel human, and it was presumed that this behaviour would reflect the birds' general perception of humans. Whilst this assumption was not tested, previous research has shown that laying hens will generalise their response to a single person to other people (Jones, 1994, Barnett et al., 1993) and their response to the researcher was indicative of their response to all humans.

The difference between white and brown strains of hen in their response to humans has been well documented in the literature, with white hens classically described as 'flighty' and the brown hens 'docile' (Jones, 1987, Murphy and Duncan, 1978). The results of the current field study agree with this description, with the white hens used on the US farms displaying much greater avoidance of humans during behavioural testing than the brown hens used in Australia. However, the fact that several stockperson behaviour variables were still included in the final linear regression models indicates that whilst genetics has a large impact on bird behaviour, the behavioural response of the hens to humans may be modified by stockperson contact. The stockperson behaviours that were predictive of bird behaviour were the amount of noise occurring in the shed (Noise / cage (log)), the amount of time spent stationary in the aisle (Stationary / cage), the number of cage approaches made by stockpeople (Approach / cage (log)), the total number of interactions that occurred in close proximity to the hens (Near cage / cage), and the maximum speed of movement of the stockpeople (Max SOM).

A high incidence of noise in the shed was associated with fewer birds at the cage front or with their heads out of the cage during testing. This was the most consistent relationship found between stockperson behaviour and hen behaviour, with noise negatively related to the proportion of birds at the cage front during testing in both the correlation and regression analyses. The majority of noise that occurred in the laying shed was associated with cleaning. Large modern sheds are cleaned by blowing the dust along the shed and out of the vents using an air hose, or occasionally a leaf blower. Cleaning with an air hose or leaf blower is an unpleasant process for both the stockperson and the hens. The cleaning equipment makes a loud noise (~ 90 dB) often for long durations at a time, and increases the level of dust in the air. Sounds at 90 dB have been shown to be aversive to laying hens (McAdie et al., 1993, MacKenzie et al., 1993), and exposure to sound at 90 dB for one hour increases both stress (heterophil: lymphocyte ratio) and fear (duration of tonic immobility) in laying hens (Campo et al., 2005). If the hens learn to associate the loud noise and increased dust in the air with humans then this could explain the positive relationship between noise in the laying shed and the fear of humans observed on-farm.

The amount of time spent stationary in the aisle, the number of cage approaches made and the number of interactions that were made in close proximity to the hens were negatively associated with fear of humans. That is, a high incidence of these behaviours was associated with more birds at the cage front or with their heads out during behavioural testing. It is likely that these stockperson behaviours are considered neutral or possibly even rewarding for the birds, allowing the birds to habituate to human presence. In a barren environment such as the laying shed, a stockperson may provide a form of enrichment for the hens (Jones, 1993), especially when their presence does not contain other aversive elements, such as loud noise or physical contact. Several handling studies have shown that visual contact is effective in reducing fear of humans (Jones, 1993, Barnett et al., 1994, Zulkifli et al., 2002), and it is not surprising that visual contact with a stationary human or a human approaching the cage but not contacting it, allowed the birds to habituate to human presence.

In addition, the stockperson behaviour variable 'Near cage' was associated with low fear of humans, as measured by the avoidance behaviour of the hens in two testing situations. This variable is a summed composite of the four types of stockperson behaviour that occurred near the hens: cage approach; cage contact; cage entry, and bird handling. The previous association between low fear of humans, time spent stationary in the aisle and the frequency of cage approaches suggests that low intensity contact with stockpeople can allow the hens to habituate to human presence. However, it was expected that stockperson behaviours such as cage entries and bird handling would be associated with greater fear of humans, as the intensity of the human stimulus is much greater during these interactions. It is therefore surprising that the 'Near cage' variable, which included the frequency of cage entries and bird handling, was associated with less fear of humans. It is likely that this relationship is due to this variable being a composite score that includes the behaviours 'Cage approach' and 'Cage contact'. When analysed separately, these behaviours were significantly associated with the behaviour of the birds, whereas the incidence of cage entries and bird handling were not. This may be because the incidence of cage entries and bird handling was quite low, and these behaviours were only likely to influence the birds directly involved in the interaction and their neighbours, who can observe the handling and close contact. Thus, while very close interactions may be fear provoking, it is possible that they did not occur often enough to influence the average flock values for the measures of fear of humans.

Another unexpected result was the association between faster maximum speed by the stockpeople and a greater number of hens putting their heads out of the cage during the Approaching Human Test, indicating low avoidance of humans. This is contradictory to the results presented by Cransberg et al. (2000), who found that the speed of movement by stockpeople in broiler sheds was correlated with increased avoidance behaviour by the birds. In addition, Breuer et al. (2000) found that the speed at which stockpeople moved their dairy cows into the dairy for milking was positively correlated with the restlessness of the cows in the presence of the stockperson. Jones (1996) lists rapidly approaching stimuli, including humans, as causing fear in poultry. Whilst this would seem intuitively correct there is surprisingly little information available in the literature to support this claim. Fear of humans in caged laying hens was reduced after daily exposure to visual contact with a human moving slowly and predictably for 17 wks (Barnett et al., 1994). These seemingly contradictory results emphasise the lack of understanding that exists in relation to the human-animal relationship, and how very specific or subtle behavioural interactions may determine the fear of humans experienced by the animals (Hemsworth, 2003). Further experimental work that specifically addresses the impact of speed of movement on the avoidance response of laying hens would be required before any conclusions can be drawn from this result.

Several features of the laying shed were associated with fear of humans, the most notable being the number of birds in the cage. A larger group size was associated with greater avoidance behaviour during the Approaching Human Test, which was not in accordance with the literature. Group size has been previously shown to decrease fear in caged laying hens when the groups were of a comparable size to those used in commercial situations (Rodenburg and Koene, 2007), although fear has been shown to increase in much larger group sizes (n = 120) (Bilcík et al., 1998). Interestingly, the proportion of birds with their heads out of the cage during the Stroll Test was negatively correlated with space allowance, so that fewer birds had their heads out when they had more floor space. This may reflect the ability of the birds to withdraw back into the cage, as greater room to move would allow the hens to withdraw more readily.

There was a tendency for birds in larger sheds to display greater avoidance behaviour. Aisle length was negatively correlated with the amount of time that the stockperson spent in the shed per cage, and it is likely that the reduced opportunities for human-animal interactions in large sheds accounted for the increase in avoidance behaviour displayed by the hens. Larger sheds were also associated with a greater incidence of noise, which was associated with greater avoidance. In addition, the behavioural differences between large and small sheds may have been influenced by the testing methodology. The Approaching Human Test was conducted on every twentieth cage in large sheds and on every tenth cage in smaller sheds. The distance between focal cages ranged between 3 - 7 m in sheds where every tenth cage was tested, and between 8 - 13 m in sheds where every twentieth cage was tested. This meant that the researcher spent more time near the birds prior to testing in the small sheds, as the focal cages were closer together. This additional time may have allowed the birds to overcome the novelty of the situation prior to the testing, and thus birds in smaller sheds displayed less avoidance behaviour than those in large sheds.

In summary, close visual contact with the stockperson was associated with less fear of humans in laying hens, whilst a high incidence of noise was associated with greater fear. Previous research on human-animal interactions in farmed species has focused on the tactile and auditory interactions that occur between stockpeople and their animals. Whilst the egg industry involves very little tactile contact between the stockpeople and the birds, this research has demonstrated that visual and auditory interactions can influence fear of humans in commercial laying hens.

1.5.3 Factors associated with the albumen corticosterone content of commercial laying hens in Australia

A high withdrawal distance was associated with lower corticosterone concentration in egg albumen collected from Australian farms. This suggests that the birds experiencing high fear of humans had lower concentrations of corticosterone circulating in their body and thus entering the egg. This result is unexpected, and in contrast to the positive relationship between fear and stress hormones reported in the literature (eg. Beuving and Vonder, 1978, Hazard et al., 2008, Fraisse and Cockrem, 2006). Research by Downing and Bryden (2002, , 2008) has demonstrated that increases in plasma corticosterone concentrations due to various stressors, including handling, were directly associated with concurrent increases in corticosterone in the egg albumen. It is therefore surprising that a behavioural measure of fear, which is presumably indicative of stress, was negatively related to albumen corticosterone concentration.

A possible explanation for this result may be that the albumen corticosterone concentration in the hens is unrelated to their response to a novel human, a presumably fear-provoking situation for fearful birds. No studies could be found that directly compared the fear responses of birds to the corticosterone concentration of their egg albumen, however it would appear that situations that are likely to elicit fear do result in an increase in corticosterone being transferred to the egg. For example, exposure to a simulated predator (a stuffed cat) for 2 hrs caused an increase in the concentration of corticosterone in the egg yolk of barn swallows, presumably due to their fear of predation (Saino et al., 2005). In addition, high concentrations of plasma corticosteroids have been associated with greater fear responses in laying hens (Barnett et al., 1994, Fraisse and Cockrem, 2006) and broilers (Hemsworth et al., 1994b), although it should be noted that these studies assessed the plasma corticosterone response of the birds to a handling stressor, rather than basal concentrations. Plasma corticosterone concentration has been positively correlated with albumen corticosterone concentration (Rettenbacher et al., 2005, Downing and Bryden, 2002), and it would appear that albumen corticosterone concentration would increase for birds that are regularly exposed to fear-provoking stimuli.

It is possible that another factor, such as the living conditions and cleanliness of the shed, was influencing the corticosterone content of the eggs. The motorised cleaning equipment used to clean the shed may have conditioned the birds to become fearful of humans, however an additional consequence of cleaning may be that the birds have a cleaner environment to live in and thus experience less stressful living conditions. The relationship between avoidance behaviour and albumen corticosterone concentration may simply reflect how often the sheds are cleaned. This potential relationship is also discussed in the section on productivity (Section 1.5.4).

A further possibility is that the behavioural variable ‘Withdrawal distance’ is not actually measuring fear of humans, and the relationship between this measure and albumen corticosterone concentration is mediated by something other than fear. However, Murphy (1978) describes withdrawal from a stimulus as indicative of a fear response, and withdrawal is a commonly used tool in assessing fear and exploration. It would seem unlikely that the birds in the current study were withdrawing or leaving their heads out of the cage due to any other motivation. It may also be possible that the withdrawal distance is measuring a different aspect of fear to that which was originally intended. For example, vigilance is also considered a measure of fear (Oden et al., 2005) and could potentially be assessed by counting how many hens had their heads out of the cage to watch the researcher as she approached during testing. If this were the case, the presence of more birds at the cage front or with their heads out during the behavioural tests would indicate high vigilance and thus high fear. The association between a low withdrawal distance (high vigilance) and high concentration of corticosterone in the egg albumen would make sense in this context. However, from personal observations during the behavioural tests this is not considered to be the case. The more flighty white birds used in the US showed substantially longer withdrawal distances, and these birds frequently panicked during the test and vocalised more often that the brown strains with lower withdrawal distances. Panic and hysteria are unequivocally indicative of fear, and these behaviours indicate that an increase in withdrawal distance is representative of increased fear.

A further comment on the validity of the results must consider the assay used to determine the concentration of corticosterone present in the egg albumen. This assay was recently developed and is not yet fully understood. For example, it is presumed that corticosterone stops entering the albumen when the egg shell begins to form, but it has not been confirmed whether the concentration of corticosterone in the albumen is a snapshot of the plasma corticosterone concentration at this time, or if it is an indicator of plasma corticosterone during the 3-4 hrs of albumen formation. However, despite these concerns, the albumen corticosterone assay has been shown to be comparable to changes occurring in basal plasma corticosterone concentrations in response to stressors, such as handling and heat stress (Downing and Bryden, 2002, Downing and Bryden, 2003). The concentration of albumen corticosterone in the present study was also negatively correlated with space allowance, with hens that had more floor space showing lower albumen corticosterone concentrations. Space allowance had been previously shown to influence stress in laying hens (Koelkebeck et al., 1987, Cunningham et al., 1987, Onbaşılar and Aksoy, 2005), and this result provides some validity to the albumen corticosterone assay used in this study.

Another possible, although less likely, explanation may be that the fear of humans was actually causing a decrease in the amount of corticosterone entering the egg albumen. This might result from the hens experiencing a series of acute stressors, causing blood to be shunted away from the shell gland. During an acute stress response, adrenaline stimulates the blood flow to be shunted away from non-essential functions, such as digestion and reproduction, and shunted toward the large muscle groups and central nervous system (Draper and Lake, 1967, Duncan and Filshie, 1979). Shunting blood away from the developing egg may result in less plasma corticosterone coming into contact with the egg, and less corticosterone diffusing into the albumen. The shunting response is part of the fight-or-flight response, and this mechanism would only be relevant if the hens were regularly exposed to acute stress during the period of albumen formation prior to the egg shell being laid down. Ovulation in laying hens occurs every 24-25 h, and the majority of albumen is laid down during the first three hours after ovulation (Whittow, 2000). Ovulation occurs 15 to 75 minutes after the previous egg has been laid (Whittow, 2000), which generally occurs in the morning for most hens. Therefore, most hens are laying down albumen and egg membranes during the morning when stockpeople are working in the shed. It would seem plausible to suggest that in sheds where hens are receiving negative interactions with the stockpeople this may result in the hens experiencing repeated acute stress responses and thus shunting blood away from the developing egg. However, exposure to a predator for two hours has been shown to increase albumen corticosterone concentrations in the eggs of barn swallows (Saino et al., 2005), and it would appear that even relatively brief acute stressors cause albumen corticosterone concentrations to increase rather than decrease. The research by Downing and Bryden (2003, , 2002) has also demonstrated that chronic stressors result in an increase in albumen corticosterone concentrations, and it appears extremely unlikely that the concentration of corticosterone in egg albumen was increased by fear of humans in the present study.

A more likely explanation is that the egg sample used for albumen corticosterone assay was biased toward eggs with low corticosterone content. For example, the eggs were collected from standard locations within the shed, and the albumen corticosterone concentration of these eggs was then compared to the average behaviour of the flock rather than the individual hens that laid each egg. Due to the inhibitive nature of stress on egg production (Moberg, 1985, Mashaly et al., 2004), stressed hens were likely to be laying less often than non-stressed hens. They were also more likely to be laying later in the day than non-stressed hens, as stress has been shown to delay the time of oviposition (Downing and Bryden, 2002, Reynard and Savory, 1999). The egg sample was collected first thing in the morning, and birds that were laying later in the day would not have been included in the sample. Thus, the chance of selecting an egg from a non-stressed hen was higher than that for a stressed hen. This would explain why the withdrawal distance was predictive of albumen corticosterone concentrations, as the greater the avoidance behaviour of the hens then the greater the potential stress levels in the flock, and the greater the bias toward selecting eggs from low-stress hens. This theory would also explain why a high incidence of noise in the shed was correlated with low albumen corticosterone concentrations, as high noise was associated with high fear of humans. However, while fear may cause a delay in the time of oviposition, fear of humans was not associated with a drop in egg production, as discussed in Section 1.5.4.

Alternately, the significant relationship between the withdrawal distance and albumen corticosterone concentration may be due to another factor that was not included in the study, or due to the low sample size (n = 20). All of the egg samples were collected from brown birds in Australia, and the relationship between albumen corticosterone and fear of humans may not be consistent for all strains of hen. It would be interesting to conduct further research in commercial situations in which both plasma and albumen samples were collected and compared to the fear of humans displayed by the hens. A more refined approach to the comparison of fear and albumen corticosterone concentration would resolve this issue, whereby the individual hens that laid each egg would also be assessed for fear of humans.

1.5.4 Factors associated with the productivity of laying hens

The most striking productivity result was that high fear of humans in the hens was correlated with greater egg production. The regression model for the number of weeks that the hens remained laying within 2% of their peak production (PHDP 2%) included one measure of avoidance behaviour; the proportion of birds with their heads out per m during the Stroll Test. Flocks that showed greater avoidance behaviour during the Stroll Test were predicted to have a high rate of lay for longer. This was an unexpected result, as fear of humans has been previously associated with reduced productive output in commercial poultry. For example, high fear of humans was associated with increased first week mortality (Cransberg et al., 2000) and reduced feed conversion efficiency in broilers (Hemsworth et al., 1994b, Jones et al., 1993), and reduced peak egg production in layers (Barnett et al., 1992). The study with laying hens by Barnett et al. (1992) used a very similar design to the current study, in which fear of humans was assessed at commercial egg farms using a variant of the Approaching Human Test. This variant of the test involved the researcher approaching the cage once rather than twice, and holding the hands in front of the body rather than in the pockets. Avoidance behaviour of the hens was assessed in the same manner, by measuring the proportion of birds at the cage front during the test. Despite the similarities in study design, the results of the field work by Barnett et al. (1992) and the current field study are contradictory. The reason for these conflicting results is not clear.

It is possible that flocks producing more eggs were experiencing greater stress due to the high metabolic demands placed on them. An animal that is already stressed will experience a heightened response to further stressors (Moberg, 1985), and thus flocks that were producing well may have been more reactive during the behavioural tests. The lack of a relationship between albumen corticosterone concentrations and egg production suggests that this is not the case, however, as was discussed in Section 1.5.3, the albumen corticosterone sample may not have been representative of the entire flock.

The relationship between light levels and PHDP 2% indicates that flocks under low light intensities could sustain a high rate of egg production for longer than flocks under brighter conditions. Egg production has been shown to increase proportionally with light intensity up to a brightness of five lux, after which there is little effect of intensity (Morris, 1967, Lewis and Morris, 1999). Around half of the sheds observed in the current study had average light levels below five lux, and it would have been expected that egg production would have been positively correlated with light intensity. However, a study by Boshouwers and Nicaise (1987) found that hen activity decreased under low light intensity, with a concurrent decrease in energy expenditure. If the dim light levels used in modern enclosed laying sheds reduced the activity of the hens to the point that they experienced improved feed conversion efficiency, this may explain the negative relationship observed between sustained egg production and light intensity.

Egg production was also predicted by stockperson behaviour. The rate of lay was greater when there was more noise occurring in the shed, and when the stockpeople had a lower speed of movement. A study by Cransberg et al. (2000), showed that low speed of movement was associated with improved productivity in commercial broiler chickens, measured in terms of first week mortality rates. This relationship in broiler chickens was attributed to fear of humans, which may have increased the chicks' susceptibility to stressors. However, as Cransberg et al. (2000) concluded, this relationship was only present in very young birds, and the birds appeared to habituate to the presence of the stockperson as they grew older. It would appear that the production benefits associated with low speed of movement were ongoing for the laying hens in the current study, although the exact mechanism for this relationship is not clear.

An explanation may lie in the type of work that the stockpeople were doing. A slower speed of movement whilst inspecting the birds would suggest that the stockperson was doing a more thorough inspection, and may be able to identify problems in the shed before they can impact on the hens. In addition, stockpeople would often clean the sheds by sweeping or blowing the dust out with an air hose or leaf blower. This would increase the amount of noise occurring in the sheds, and markedly slow down their speed of movement along the aisle. Whilst the relationships between speed of movement and the tasks being performed by the stockpeople were not investigated, it is possible that more noise and a lower average speed of movement were associated with a greater proportion of the stockperson's time spent cleaning. Poultry produce more dust than any other intensive farming situation (Takai et al., 1998), and require regular cleaning to avoid dust build up. This dust has been shown to carry high concentrations of micro-organisms that can cause disease in both stockpeople and the hens (Seedorf et al., 1998, Marois et al., 2002). A clean shed would have obvious benefits for the health of the birds, and these benefits may have translated into greater egg production in the sheds observed.

High fear of humans was also corelated with improved productivity. It may be possible that the fear of humans experienced by the hens initiated a mild stress response, and that this mild stress response actually enhanced egg production. A study by Taylor (2001) showed that hens that did not have control over their food and lighting regime laid more eggs than those that did have control. The author concluded that mild exposure to stressors may increase reproductive capacity, and cites several relevant studies that support this notion. For example, Ramaley (1981) demonstrated that exposure to heat stress delayed maturation in immature mice, but enhanced fertility and litter size in mature cycling adults. Unfortunately, no physiological data were presented to support the theory that mild stress was experienced by the hens in the study by Taylor (2001), and it would seem more likely that another factor that was not included in the study was responsible for this anomaly. This factor may be related to the cleanliness of the laying shed. As discussed above, noise was strongly correlated with avoidance behaviour in the hens, and was also associated with the loud cleaning equipment used to blow the dust out of the sheds. A high amount of noise in the shed may result in both fearful birds and a cleaner environment, and could result in an improvement in egg production despite the fear that the birds are experiencing due to the improved living conditions.

The age that the bird reached peak egg production was greater in sheds where the stockpeople had a higher maximum speed of movement and spent more time in the ends of the aisles, although only maximum speed was included in the final regression model. This result is similar to the relationship found between peak production and the average speed of movement by the stockperson discussed above, in which faster speeds of movement were associated with poorer peak production. Delays in sexual maturity have been associated with greater levels of fear in laying hens (Craig et al., 1983), and it is tempting to presume that high speed of movement is fear provoking for the hens, which resulted in a stress response that delayed sexual maturity. However, the positive relationship between speed of movement by the stockperson and the number of birds with their heads out of the cage during the Approaching Human Test indicates that high speed of movement did not cause the birds to be fearful of humans. The mechanism by which speed of movement is influencing the hens is not apparent from the results of this study.

More time spent in the ends of the aisles by the stockperson was associated with higher mortality rates, and was correlated with a delayed age at peak production. The amount of time that stockpeople spent in the ends of the aisles was usually spent cleaning and maintaining the equipment used for automatic feeding, egg collection and manure removal. Spending more time maintaining this equipment would benefit the hens by ensuring that they regularly received feed and had their manure removed. However, whilst the stockpeople were working in the ends of the aisles they often made a lot of noise, either due to cleaning or simply moving the equipment around. Noise was shown to be strongly related to the fear of humans that the birds experienced, and this additional work in the ends of the aisles may have contributed to their fear of humans. In addition, whilst the noise made at one end of the shed cannot be heard by the hens at the other end of the shed, the silhouette of the stockperson is clearly visible against the well-lit area at the front of the shed to all of the hens in that aisle. A study by Jones et al. (1981) showed that hens of a brown medium weight strain experienced a fear response to a human standing in a corridor at a distance of 30 m. It's possible that seeing a human from a distance, often in association with loud noise, was fear provoking for the hens in the current study, and this increase in fear was associated with the increased mortality and a delay in peak production. However, there were no significant relationships between the avoidance behaviour of the hens and their mortality or age at peak production.

An alternative, albeit more mundane, explanation may simply be related to the strain of bird and the type of shed they were housed in. The modern sheds with manure belts were only observed in Australia, and these sheds required regular maintenance in the ends of the aisles to clean the manure belts, as well as maintaining the automated systems and cleaning the air filters used for drying the manure. The modern Australian sheds also housed the brown laying strains, which have higher peak production rates as well as higher mortality rates, according to their breed standards. The observed relationships between time spent in the ends of the aisles and mortality may simply be a product of keeping bird strains with high mortality rates in modern facilities. The relationship between the age at peak production and the amount of time spent in the ends of the aisles cannot be explained by these variables.

The average egg weight was predicted to be greater in Australia, and when the stockpeople made few entries into the cages. The brown strains used in Australia may partially explain this, as they lay heavier eggs than the white strains. The relationship between egg weight and cage entries does suggest a relationship between fear of humans and productivity, as an increased incidence of extremely close contact with the stockperson is likely to be aversive for the birds. High fear of humans has been associated with reduced egg weights in experimental situations (Bredbacka, 1988, Hemsworth and Barnett, 1989), however egg weight was not associated with hen behaviour in this study. An alternative explanation is that stockpeople often made cage entries to inspect a sick bird or remove dead birds. A low incidence of these behaviours may indicate a healthy flock that is producing well and laying heavier eggs.

In summary, the productivity of commercial laying hens was associated with fear of humans in the hens, light levels in the shed and stockperson behaviour. The laying hens produced more eggs when there was more noise occurring in the shed, when the stockpeople moved slowly, when the light levels were low and when the hens displayed greater fear of humans. The positive relationship between fear of humans and egg production was unexpected and cannot be explained with the evidence at hand. It is possible that the relationship between fear of humans in the hens, stockperson behaviour and improved production is related to the cleanliness of the laying shed.

1.5.5 Summary and conclusions

The aim of this research was to determine whether a sequential relationship exists between stockperson attitudes, stockperson behaviour, fear of humans in laying hens and hen productivity on commercial farms in Australia and the USA. The results of this research demonstrate that stockperson attitudes predict stockperson behaviours on egg farms, and that stockperson behaviour predicts avoidance behaviour in laying hens. However, avoidance behaviour in laying hens was found to predict the corticosterone content of egg albumen and the productivity of the flock in the opposite direction to what was expected. A summary of these relationships are presented diagrammatically in Figure 2. It should be reiterated that these relationships are observational and should not be interpreted as causal, however they do provide a basis for further experimental work which could determine causality.

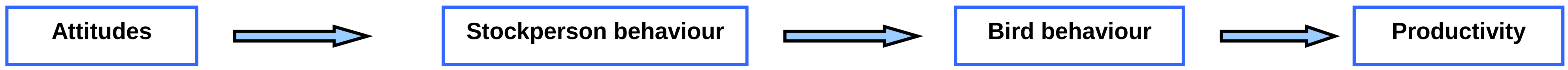
Several relationships between stockperson behaviour and attitudes were established. Poor stockperson attitudes were associated with more noise, faster speed of movement, more time in the shed, less time spent stationary, less time at the start of the shed and less time in the ends of the aisles. Stockperson attitudes were also correlated with the number of people working in their shed, with poorer attitudes associated with more stockpeople.

Several stockperson behaviours were associated with fear of humans in commercial laying hens. Hens that were exposed to a high incidence of noise displayed greater fear of humans, whilst hens that experienced a high incidence of close visual contact and high speed of movement displayed less fear of humans. From these results it can be seen that three of the stockperson behaviours that were important to the hens were also predicted by the attitude subscales. In addition, the attitude subscales were also correlated with the avoidance behaviour of the hens, with positive attitudes correlated with less avoidance behaviour. This supports the hypothesis that attitudes are influencing the way that stockpeople are interacting with their hens, and these interactions are influencing the fear of humans that the hens experience. Country and bird strain also contributed significantly to the prediction of bird behaviour, which was as expected due to the large differences in behaviour that was observed between the brown strains used in Australia and the white strains used in the USA.

The relationships between bird behaviour, corticosterone content of egg albumen and productivity were not in the expected direction. Greater avoidance behaviour was associated with lower corticosterone concentration in egg albumen and improved egg production. The negative relationship between fear of humans in the hens and albumen corticosterone content may be due to fear of humans causing the egg sample to be biased toward eggs with low corticosterone content, and thus not being representative of the entire flock. The negative relationship between fear of humans and improved egg production was unexpected, and is not in accordance with the literature. It is possible that this result is due to the cleanliness of the laying shed improving the health and productivity of the hens, and that these benefits outweigh the negative impacts of fear caused by the cleaning process.

In conclusion, the findings of this research demonstrate that significant relationships exist between stockperson attitudes and stockperson behaviour, stockperson behaviour and fear of humans in laying hens, and fear of humans in laying hens and productivity in the egg industry. These results suggest that a human-animal relationship does exist in the egg industry, albeit with some relationships in unexpected directions. Further exploration of this relationship is required to determine causality, and this work is continued in the following experimental chapters.

Model for human-animal interactions in the livestock industries



Findings for laying hens in the egg industry

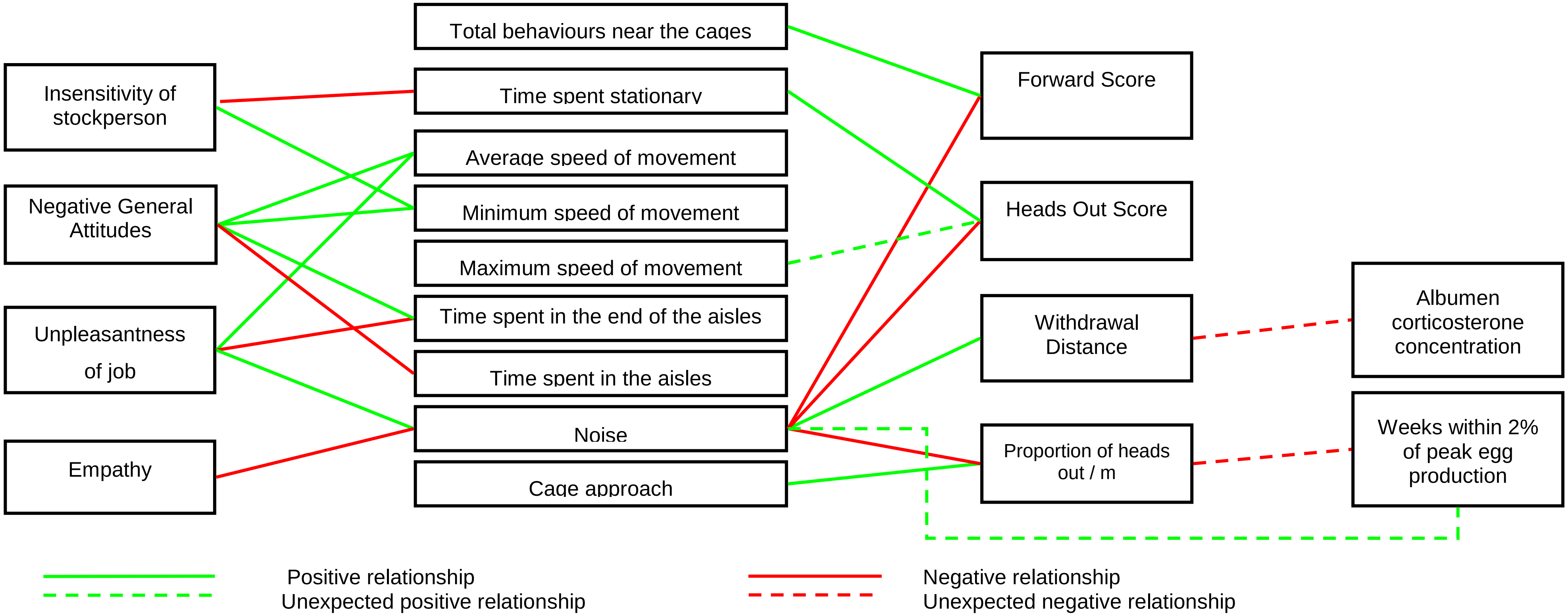


Figure 2. A diagrammatic summary of the main findings of the field work in relation to the human-animal interactions model

Chapter 2: The impact of duration and proximity of human contact on fear of humans in commercial laying hens (Experiment 2)

2.1 Introduction and aims

The effects of human contact were examined in a commercial setting. The benefits of this were two-fold. Firstly, a commercial egg farm allows immediate access to large numbers of laying hens, substantially increasing the sample size. Secondly, for the research to be of benefit to the welfare of laying hens, it must be relevant within a commercial setting. Conducting the experiment under commercial conditions will facilitate this process.

Whilst imposing tactile human contact to reduce fear of humans in laying hens may be suitable in an experimental situation, handling large numbers of birds on a regular basis is neither practical nor appropriate in a commercial setting. Also, tactile contact may not be the most appropriate method of reducing fear of humans in laying hens. In the field study (Chapter 1), the stockperson behaviours that were associated with reduced fear of humans involved visual contact at close proximity, rather than tactile contact. Visual contact has been shown to be equally effective in reducing fear of humans in poultry when compared directly to tactile contact, if not more so (Jones, 1993, Zulkifli and Siti Nor Azah, 2004). Thus, visual human contact treatments were deemed sufficient for the purposes of this experiment. Two aspects of visual contact were examined; the duration of contact and the proximity of contact. The field work reported in the previous chapter found that an increased amount of time spent stationary by the stockperson and more stockperson behaviours performed close to the cages were associated with lower fear of humans in laying hens. These results indicate that proximity and duration of human contact are an important determinant of fear of humans in laying hens, and warrant further study.

This experiment also formed a preliminary study to identify human contact that reduces fear of humans in hens for use in future experimental work (Chapter 3). Thus, the aim of this present experiment was to determine whether daily visual contact with a human at various proximities and durations could reduce the avoidance response and plasma corticosterone response to handling in commercial laying hens.

2.2 Materials and Methods

2.2.1 Experimental design and location

This experiment was conducted in four environmentally-controlled laying sheds at a large commercial farm in central Victoria, Australia. Each shed contained three rows of cages, creating four aisles. Details of the sheds are presented in Table 25. All sheds were equipped with fully automated feed, water, egg collection and manure removal systems.

Table 25 Characteristics of the commercial sheds used during Experiment 2

	No. birds/shed	No. birds/cage	Cage width (cm)	Aisle length (m)	Age of birds (wks)
Shed 9	22 000	6	49	74	63
Shed 10	22 000	6	49	74	31
Shed 11	19 600	6	49	53	50
Shed 12	26 000	8	70	76	69

Human contact was imposed on the laying hens for one of three durations (2 s, 30 s or 90 s), at one of three distances from the cage front (0 cm, 75 cm or 150 cm). This created a total of nine treatments in a 3x3 factorial design.

The experimental cages were located in the second tier of cages (~ 1m in height) at evenly spaced intervals along one side of each row. The intervals were staggered between rows to prevent birds in one experimental cage being able to see directly through the row to the experimental cage in the adjacent row. The side of the row that the experimental cages were located on was alternated between sheds so that Sheds 9 and 11 had the experimental cages on the left side of the rows and Sheds 10 and 12 had the experimental cages on the right side of the rows. This design was used to allow all locations in the shed to be sampled, avoiding any location specific effects on the birds' response to humans.

For blocking purposes each row of cages was divided in half. This created two blocks of nine treatments in each row. Due to shed differences in cage widths (see Table 25, previous) the distance between experimental cages varied slightly, with a 3.4 m interval (seven cages) between experimental cages in Sheds 9, 10 and 11, and a 3.5 m interval (five cages) in Shed 12. Each shed contained three rows of cages, which created a total of six treatment replicates per shed (n = 54 cages per shed). The experimental design in one shed is presented in Figure 3.

2.2.2 Human contact treatments

Treatments were imposed for 28 days (Day 2 to Day 29) between 0800 h and 1300 h on weekdays, and between 0900 h and 1700 h on weekends. The treatments took approximately 1 h per shed to impose. The researcher imposed the treatments while standing directly in front of the experimental cage, looking directly at the birds in that cage. The treatments were timed using a stopwatch, and all movements were made in a slow and predictable manner to avoid startling the hens. The direction of movement through the shed was randomised daily so that the direction of approach within the aisle and order in which the aisles were visited was different every day. The researcher dressed in a standard manner for the daily treatments, wearing blue overalls and yellow gumboots. This attire was unfamiliar to the birds, as the stockpeople dressed in casual attire whilst at work.

The following treatment combinations were imposed for 28 days:
1/ Visual contact with a stationary human for one of three durations (2 s, 30 s or 90 s)
2/ Visual contact with a stationary human at one of three proximities from the cage (0 cm, 75 cm or 150 cm)

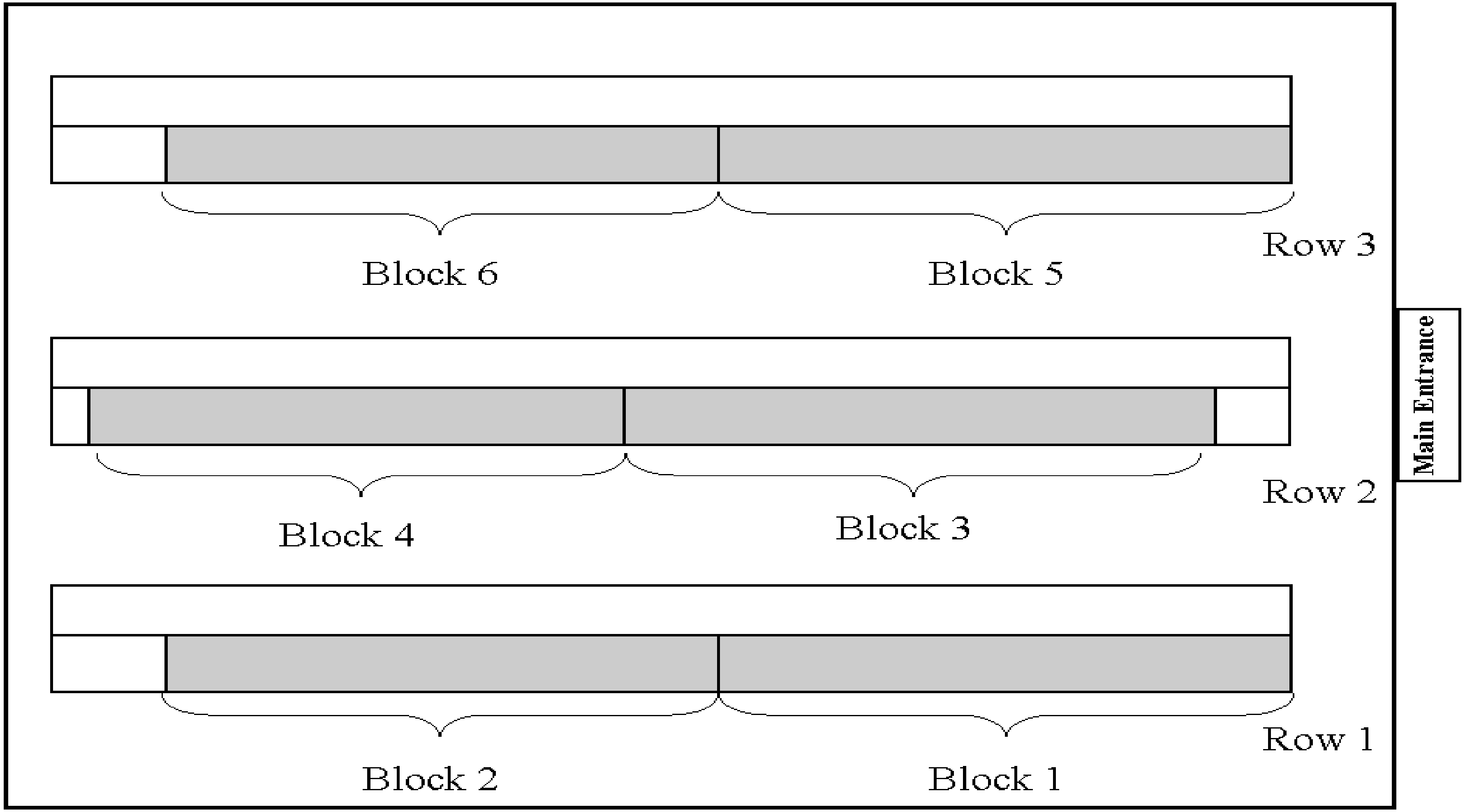


Figure 3. A diagram of the experimental design in one of the commercial sheds used in Experiment 2

2.2.3 Behavioural assessment

Birds were tested for fear of humans at the beginning (Day - 5), middle (Day 15) and end (Day 30) of the experiment using the Approaching Human Test and the Stroll Test. The tests were administered in the same manner as in the field study (described in the Chapter 1 methodology section), however the Stroll Test was adapted for use with specific focal cages. During the test the researcher walked along the aisles at a standard speed (one step / s), filming the birds in the second tier containing the treatments cages in the same manner as for the field study. During video analysis the number of birds with their heads out of each focal cage was counted as the researcher approached within a one cage distance of the treatment cage. This resulted in the variable "Proportion of Heads Out', with a higher proportion indicating lower fear of humans.

The Approaching Human Test was administered to the treatment cages in consecutive order along each aisle, and the aisle order was randomised within each shed. Prior to each test commencing, the researcher walked slowly along the aisle and stopped adjacent to the treatment cage to be tested. The researcher stood stationary in this position for 5 s, with her hands in her pockets for the entire test. This allowed the birds in the treatment cage to adjust to the presence of the researcher, reducing the possibility of startling the birds as the test commenced. An assistant waited at a distance of 1 m from the researcher and timed the test in the following manner: after 5 s of the researcher waiting next to the treatment cage the assistant verbally notified the researcher to commence the test. The researcher then stepped sideways so that she was standing directly in front of the treatment cage but on the opposite side of the aisle. After 5 s the researcher stepped forward, standing directly in front of the treatment cage with her torso contacting the feed trough. After a further 5 s the researcher stepped backward across the aisle and stood opposite the treatment cage. After 5 s the researcher stepped forward again, to the position directly in front of the treatment cage. The total test took 20 s, consisting of two 5 s periods with the researcher standing on the opposite side of the aisle and two 5 s periods with the researcher standing directly in front of the cage. During each 5 s period the researcher counted the maximum number of birds that put their head into the front 5 cm of the cage, the number of birds that put their head out of the cage. At the end of each 5 s period the researcher made a final point count of the number of birds that still had their heads in the front 5 cm of the cage. At the end of the AHT the researcher had recorded 3 variables, measured every 5 s for 20 s. The resulting twelve variables were all significantly correlated with each other for each test day (Day - 5, $P \leq 0.02$, Day 15, $P \leq 0.00$, Day 30, $P \leq 0.00$), and were thus deemed sufficiently similar to convert to a single value for each treatment cage. All twelve values for each focal cage were converted to z-scores and summed to create a single behavioural value (SumZ). A high value for the variable 'SumZ' indicates low fear of humans.

The number of birds in each focal cage was counted on each of the behavioural testing days (Days - 5, 15 and 30). This allowed the proportion of birds forward in each of the cages to be accurately calculated for each test day by comparing the results of the behavioural tests to the number of birds in each cage. The number of birds in each cage had to be re-counted regularly as some cages experienced mortalities during the experiment, and thus using the original bird count (Day - 5) would have underestimated the proportion of birds forward for those cages that had experienced mortalities. Treatments were not imposed on the testing days.

2.2.4 Physiological assessment

Six birds from each treatment (n = 54) were blood sampled on Day - 5 and Day 30 of the experiment to assess the hens' corticosterone response to 4 minutes of restraint by a human.

All birds were sampled from one shed (Shed 10), with one bird sampled from each experimental cage. Shed 10 had three rows of cages, and all three were sampled simultaneously. The treatment cages were sampled in sequential order from the front to the back of the shed. The cages were sampled sequentially to avoid exposing unsampled birds to close visual human contact prior to blood sampling. Three people caught and restrained the birds while two people collected the blood samples. All birds were sampled after 1300 h to reduce the effects of the diurnal increase in plasma corticosterone concentrations that occur in relation to egg laying (Beuving and Vonder, 1977).

One bird from each treatment cage was selected based on her position in the cage as the researcher approached. That is, a bird that was standing at the front of the cage when approached was selected from the first cage, a bird standing in the middle of the cage would be selected from the next treatment cage, and finally a bird standing at the back of the cage would be selected from the third treatment cage. This selection order was repeated along the aisle for all eighteen treatment cages. Once a bird was selected based on her position in the cage, the researcher caught only that hen. This avoided consistently selecting hens that were positioned at the cage front, and thus avoiding unintentionally selecting birds that may have a particular behavioural disposition. For example, birds at the cage front are closer to the feed trough and so a dominant hen would be positioned near the feed trough more often (Keeling, 1995, Mankovich and Banks, 1982). An unintentional bias toward selecting more dominant hens could potentially confound the corticosterone response to human contact if dominant hens responded in a different manner to submissive hens.

Once caught, the hen was gently removed from the cage and held upright under the handlers arm. Once the bird was in this position the handler started a stopwatch and began slowly walking (on step / s) toward the start of the shed. The handler walked in this manner for two minutes, walking along the non-treatment aisle to avoid walking near non-sampled experimental birds. After two minutes the handler then sat quietly at the start of the shed near the blood sampling equipment and waited for a further two minutes. The birds were handled in this manner to standardise the handling that each bird received. The birds were held for a total of four minutes, as this duration has been previously demonstrated by Beuving and Vonder (1978) to be a sufficient amount of time to cause a substantial increase in plasma corticosterone concentrations.

After four minutes of handling the birds were placed on their side on a table with their wing extended. An experienced blood sampler then withdrew a blood sample of 4-5 ml from the wing vein, using an EDTA coated syringe. The birds were then replaced in their original home cage and the handler moved to the next treatment cage along in the row. As the birds were not marked when they were replaced in the cage, it is possible that some birds that were sampled on Day - 5 were repeat sampled again on Day 30. The blood samples were stored on ice until they could be centrifuged later that day. The plasma fraction was removed, frozen, and stored at -20C. The plasma was later thawed and analysed by a third party using a competitive enzyme immunoassay (EIA) kit from IDS Ltd. (Fountain Hills, AZ, USA).

2.2.5 Statistical analysis

Behavioural assessment

A repeated measures analysis of variance was used to examine the effects of the human contact treatments on bird behaviour. Bird behaviour was measured using the following two variables: the sum of the twelve z-scores obtained for each focal cage during the AHT (SumZ), and the proportion of heads out of each focal cage during the Stroll Test (PropHO). The pre-treatment data collected on Day - 5 for the AHT and Stroll Test were significantly correlated with the data collected on Days 15 and 30, and were thus used as covariates for the SumZ (AHT) and PropHO (Stroll Test) analyses. A univariate analysis of variance was used to detect significant differences between sheds for each behavioural variable. Comparisons between pairs of means were tested using the post-hoc Scheffe tests for the repeated measures analyses, and the post-hoc LSD tests for the ANOVAs.

Physiological assessment

A repeated measures analysis was conducted to examine the effects of the human contact treatments on the plasma corticosterone response to handling of the hens. Whilst the majority of samples taken on Day 30 would have been from different birds to those samples taken on Day -5, a repeated measures analysis was considered appropriate as it was the cage, rather than individual birds, that was the experimental unit.

2.3 Results

2.3.1 Behavioural assessment

Approaching Human Test

A univariate ANOVA found significant (P < 0.01) differences between sheds for the summed Z scores on each testing day. Shed was thus included as a factor in further analyses.

A repeated measures analysis was conducted using Test Day, Shed, Duration and Proximity as factors. The results are presented in Table 26. Pre-treatment data (Day - 5) were used as covariates for the Day 15 and Day 30 data. There was a significant effect of Proximity (F = 3.56, P = 0.03), with birds in the 0 m treatments showing the least avoidance behaviour. A post-hoc Scheffe test determined that the 0 m treatments were significantly less fearful than those in the 1.5 m treatments (P < 0.05).

There was also a significant Test Day x Shed interaction (F = 5.67, P = 0.00), presented in Table 27. The birds in Shed 10 showed a significant (P < 0.01) decrease in avoidance behaviour from Day 15 to Day 30, whilst the birds in Shed 11 showed a significant (P < 0.01) increase in avoidance during the same period. The birds in Shed 10 also displayed significantly (P < 0.05) less avoidance than all other sheds on Day 30. There were no other significant effects of time or treatment.

Stroll Test

An analysis of variance detected significant (P < 0.01) differences between sheds on every testing day for the Stroll Test, with the birds in Shed 12 consistently displaying greater avoidance than the birds in Sheds 9, 10 and 11 for the duration of the experiment. In fact, when placed in order of most fearful to least fearful the sheds followed the same pattern on each test day.

Table 26 Treatment effects for the adjusted mean (± SEM) results for the Approaching Human Test (SumZ) and Stroll Test (PropHO) conducted during Experiment 2

	Duration					Proximity					Test Day					
	2 s	30 s	90 s	F	p	0 m	0.75 m	1.5 m	F	p	Covariate Day -5	Day 15	Day 30	F	p	df
SumZ	-1.06	1.07	0.48	1.89	0.16	1.83[a]	-0.26	-1.07[b]	**3.56**	**0.03**	-0.00	0.23	0.10	0.05	0.82	3, 172
	(0.80)	(0.80)	(0.79)			(0.79)	(0.81)	(0.81)				(0.54)	(0.54)			
Prop HO	0.33	0.34	0.30	1.37	0.26	0.32	0.33	0.33	0.09	0.91	0.27	0.33	0.32	0.04	0.84	3, 177
	(0.02)	(0.02)	(0.02)			(0.02)	(0.02)	(0.02)				(0.01)	(0.01)			

Significant differences within a row based on a post-hoc Scheffe test are depicted by different letters, [abc] P < 0.05

Table 27 Test Day x Shed interactions for the adjusted mean (± SEM) SumZ value obtained during the Approaching Human Test in Experiment 2

	Shed 9	Shed 10	Shed 11	Shed 12
Day - 5	-0.13	0.20	1.36 ^{a}	-0.53 ^{b}
	(1.09)	(1.07)	(1.06)	(1.09)
Day 30	0.82 $^{x\,a}$	0.27 $^{x\,b}$	-2.36 ^{z}	-0.78 ^{xy}
	(1.08)	(1.06)	(1.05)	(1.18)

Significant differences within rows based on a post-hoc Scheffe test are depicted by different letters, ^{xyz} P < 0.01 ^{abc} P < 0.05, df = 6, 172

A repeated measures analysis was conducted for the Stroll Test using Test Day, Shed, Duration and Proximity as factors. Pre-treatment data (Day -5) were used as covariates. The results are presented in Table 26. No significant (P < 0.05) effects of Test Day, Duration or Proximity were found for the proportion of heads out during the Stroll Test (Prop HO).

2.3.2 Physiological assessment

The results of the repeated measures analysis for the plasma corticosterone (ng/ml) response to handling are presented in Table 28. Significant differences were detected for the Proximity treatments (F = 6.25, P = 0.00), with birds in the 0 m treatments having a greater plasma corticosterone response to handling than birds in the 0.75 m (P < 0.01). A significant Bleed Day x Proximity interaction also occurred (F = 3.59, P = 0.04), and is presented in Table 29. The plasma corticosterone response to handling for birds in the 0 m treatment group was significantly (P < 0.01) higher than the other groups on Day - 5, prior to the treatments commencing. By Day 30, the corticosterone response for birds in the 0 m group had dropped to similar levels as the birds in the 0.75 m and 1.5 m groups, and was not significantly different. It is likely that the significant main effect of Proximity is due to the large decrease in the plasma corticosterone response shown by birds in the 0 m treatment. In addition, the birds in the 0.75 m treatment group showed a significant (P < 0.05) increase in plasma corticosterone response.

Table 28 Treatment effects for the adjusted mean (± SEM) plasma corticosterone concentrations in response to handling for birds in Experiment 2

	Duration					Proximity					Bleed Day			
	2 s	30 s	90 s	F	p	0 m	0.75 m	1.5 m	F	p	Bleed 1	Bleed 2	F	p
Corticosterone concentration (ng/ml)	2.31	2.52	2.23	0.67	0.51	2.81^{x}	2.01^{y}	2.25	6.25	0.00	2.38	2.39	0.06	0.80
	(0.16)	(0.16)	(0.16)			(0.16)	(0.16)	(0.16)			(0.14)	(0.15)		

Significant differences within rows are depicted by different letters ^{abc} P < 0.05 ^{xyz} P < 0.01, df = 2, 45

Table 29 Bleed Day x proximity interactions for adjusted mean plasma corticosterone response (± SEM) to handling for birds in Experiment 2

	Bleed 1			Bleed 2				
	0 m	0.75 m	1.5 m	0 m	0.75 m	1.5 m	F	p
Corticosterone concentration (ng/ml)	3.26^{x}	$1.78^{y\,a}$	2.10^{y}	2.35^{y}	$2.24^{y\,b}$	2.40^{y}	3.59	0.04
	(0.26)	(0.26)	(0.26)	(0.26)	(0.26)	(0.26)		

Significant differences within rows are depicted by different letters ^{abc} P < 0.05 ^{xyz} P < 0.01, df = 2, 45

75

2.4 Discussion

Fear of humans was significantly reduced for birds receiving visual human contact at 0 m. Birds that had received this treatment remained at the cage front more often when approached by the researcher, and showed a significant decrease in the plasma corticosterone response to handling, indicating a reduction in fear of humans. These results agree with the behavioural findings of the field research (Chapter 1), in which stockperson behaviours that occurred close to the cages were associated with less fear of humans.

Significant differences in avoidance behaviour were recorded between the four sheds. The birds in Shed 12 showed a roughly linear reduction in fear during the course of the experiment for both measures of behaviour, whilst the other sheds displayed large variation in their responses. It is possible that the birds in Shed 12 were responding to the treatments in a different manner to that of the other sheds. The birds in Shed 12 were also consistently more fearful for both measures of behaviour throughout the experiment. Similarly, the birds in Shed 10 were consistently less fearful. This is demonstrated particularly well by the Stroll Test, in which the order of sheds from most fearful to lest fearful was Sheds 12, 9, 11 and 10. This shed order also represents the ages of the birds, from the oldest in Shed 12 to the youngest in Shed 10. It is possible that the age of the birds may be influencing the way that they respond to the human contact treatments. This is in accordance with the results of a study by Jones (1995), who found that non-handled chicks became more fearful of humans as they aged. The sheds were also maintained by different stockpeople, perhaps leading to variation in avoidance behaviour. In addition, the birds in Shed 12 were housed in larger groups per cage than those in Sheds 9, 10 and 11. Large group size was associated with increased avoidance in Chapter 1, and may have been influencing the response of the birds in this study. Thus, it would appear that the differences between sheds may be due to a number of factors, such as the age of the birds, variation in human contact and the housing conditions of the shed. These factors may have altered the rate at which the hens in different sheds habituated to the researcher, particularly as the experiment was conducted over a relatively short period (30 days).

The birds receiving human contact at close proximity (0 m) showed a significant decrease in avoidance behaviour during the Approaching Human Test. This is also in accordance with the results of the field study (Chapter 1), in which human behaviours that occurred within close proximity to the cage were associated with reduced avoidance behaviour. However, it was unexpected that the human contact at a medium proximity (0.75 m) did not reduce avoidance behaviour in the present experiment. A possible explanation for this may relate to the visibility of the researcher's eyes, as this has been previously reported to increase fear and avoidance behaviour in domestic fowl (Scaife, 1976a, Scaife, 1976b, Jones, 1980a, Rosa Salva et al., 2007). The researcher's eyes were clearly visible to the birds in the 0.75 m and 1.5 m treatments, but were only visible to some of the birds in the 0 m treatment. This occurred because the researcher was standing with her torso against the feed trough at the front of the cage. If the hens were standing at the back of the cage, the manure belt above them blocked their view of the researchers face, leaving only a clear view of the researcher's body. Birds at the front of the cage were still able to look up and make eye contact; however, an important element of the situation was that they were also able to move out of eye contact if they wished. The birds receiving the 0.75 m and 1.5 m treatments were unable to escape the gaze of the researcher, and may have perceived the treatments as more threatening than the birds in the 0 m treatments. This may have delayed their habituation to the researcher in comparison to the birds receiving the 0 m treatments, and so displayed greater avoidance behaviour when tested.

The plasma corticosterone response to gentle handling increased for the birds receiving the 0.75 m treatments, but was significantly reduced for birds receiving the 0 m treatments. The birds in the 0 m treatments started the experiment with significantly elevated corticosterone responses (D ay -5), which then dropped to the same levels as the birds in the other treatments by the conclusion of the experiment. These pre-treatment differences in corticosterone response cannot be explained, as the birds were allocated to the treatments at random. Therefore it is not clear as to whether the significant drop in corticosterone response seen in the 0 m birds on Day 30 was due to the Proximity treatments or simply due to the birds ‘returning to normal’. The fact that the 0 m treatment was also associated with a reduction in avoidance behaviour indicates that this change was due to a treatment effect.

The close proximity (0 m) treatment was the only human contact treatment to reduce fear of humans in commercial laying hens. Whilst it is impractical to regularly impose this type of human contact in a commercial situation, it may be useful for stockpeople to be aware of their proximity to the hens whilst carrying out their work and moving through the shed. For example, they may consider walking along one side of the aisle rather than walking down the middle so that they are closer to the birds.

In conclusion, visual contact with a stationary human at close proximity (0 m) significantly reduced the avoidance behaviour and plasma corticosterone response to handling in commercial laying hens. The duration of human contact had no effect on avoidance behaviour or corticosterone response.

Chapter 3: The effects of human proximity and handling on the behaviour, physiology and productivity of laying hens (Experiment 3)

3.1 Introduction

The experiment reported in Chapter 2 explored the impact of human contact on the fear responses of caged laying hens. This experiment demonstrated that the proximity of visual human contact affected fear of humans. Although Experiment 2 demonstrated the potential for reducing fear in commercial situations, the benefits of the treatments on production variables could not be studied due to the automated nature of commercial egg collection. In addition, this previous experiment was only conducted for one month and utilised birds aged between 31 and 69 weeks, which may have had well-established learned fear responses to humans at the commencement of the experiment. The current experiment examined the long term impacts of varying human contact on the welfare of laying hens.

A number of studies with poultry have previously demonstrated the link between human behaviour and fear of humans, and how this influences the behaviour, welfare and productivity of poultry. The vast majority of these studies have used the following methods to successfully reduce fear of humans: tactile human contact, such as gentle restraint, stroking, or placing in a box (Hughes and Black, 1976, Jones, 1994, Jones, 1995, Jones, 1993, Jones and Faure, 1981); visual human contact, such as standing in front of the cage or placing a hand in the cage without touching the birds (Zulkifli et al., 2002, Jones, 1993, Barnett et al., 1994, Reed et al., 1993, Hemsworth et al., 1994b); or a combination of both, such as allowing birds to observe other birds being handled in a gentle manner (Zulkifli and Siti Nor Azah, 2004, Jones, 1993). These methods have also been successfully combined with auditory contact (gentle talking) and feeding (Jones, 1993, Graml et al., 2008) to reduce fear of humans in poultry.

When compared directly, visual human contact was found to be more effective than tactile contact (gentle stroking) in reducing avoidance behaviour in layer chicks in one experiment, and was equally as effective as tactile contact in reducing avoidance behaviour and the tonic immobility response in a second experiment (Jones, 1993). The author proposed that visual contact allows habituation to human presence, which can then generalise to include subsequent physical contact and restraint, such as that incurred during tonic immobility testing. A separate study by Zulkifli and Nor Azah (2004) compared pleasant visual and tactile contact as a means of reducing fear of humans and improving production in broiler chicks. Pleasant tactile contact consisted of all birds being held and gently stroked for 30 s on a daily basis, while pleasant visual contact consisted of all birds watching another bird being gently held and stroked for 10 minutes daily. When the treatments were compared, the authors found that both treatments caused a substantial and equal reduction in the tonic immobility response of broiler chicks, and the authors concluded that visual and tactile human contact were equally capable of reducing fear of humans, although only the pleasant tactile contact resulted in improved growth parameters (Zulkifli and Siti Nor Azah, 2004). No other studies have been identified that directly compare the fear-reducing effects of visual and tactile human contact on domestic fowl, however the current examples agree that visual contact of a positive nature is at least as effective in reducing fear of humans in poultry as tactile contact. In light of this evidence, visual human contact was used in the current experiment, rather than the more labour intensive tactile contact.

A large body of literature also exists that examines the impact of human behaviour on the physiological and production parameters of poultry, and these studies were often conducted in conjunction with measures of fear of humans. Similar to the results of the studies mentioned above, human contact of a visual, tactile or auditory nature have been successfully used to improve productivity (Jones and Hughes, 1981, Zulkifli and Siti Nor Azah, 2004, Collins and Siegal, 1987, Barnett et al., 1994) and immune function (Gross and Siegel, 1982, Zulkifli et al., 2002, Gross and Siegal, 1979) in poultry. However, the vast majority of these studies used chicks or young birds, and treatments were imposed for a relatively short period of time. For experiments using broilers, the handling treatments were imposed for a maximum of six weeks, as this is a common age at which birds are slaughtered. To examine the impact that handling treatments have on egg production in layers, the birds must first reach sexual maturity. This usually occurs from eighteen weeks of age. However, very few experimental studies have used adult laying hens, and only two have administered the handling treatments for an extended period of time. The first study, conducted by Hughes and Black (1976), imposed tactile handling treatments from two days to 35 weeks of age and recorded the changes in avoidance behaviour, egg production and egg shell quality that occurred. The handling treatments consisted of two stages related to the age of the birds. Up to 16 wks of age the birds were caught daily and placed in a box as a group. From 18 to 35 wks of age the birds were simply caught and held for a few seconds daily. Non-handled birds were housed in the same room but visually separated from the handling treatments by black curtains. Whilst there were no treatment effects on egg production, the handled birds had a higher occurrence of egg shell deformities. The authors attributed this increase to the contraction of the oviduct that occurs in response to adrenaline, which was presumably released during the handling treatments. It was concluded that regular handling was a mild stressor, but not one of sufficient strength to inhibit egg laying unless the birds were unfamiliar with it. The second study, conducted by Barnett et al. (1994) imposed visual human contact of a positive nature daily for 17 weeks on laying hens that were 19 weeks old. This additional human contact, which involved slow and deliberate movements, resulted in a 6% improvement in egg production when compared to that of the control birds that received minimal human contact. If these positive human-animal interactions could be successfully transferred to commercial production units, both hen welfare and financial outcomes could be improved.

Due to the scarcity of similar long-term studies, the current experiment was designed to examine the long term impact of a variety of handling treatments on the behaviour, physiology and production of laying hens. These handling treatments were designed to represent both positive and negative human contact; however as the results of Experiment 2 have shown that the proximity of the human contact can be an important factor, the human contact treatments were also imposed at varying distances from the birds. Only one study was identified that quantified the distance between the experimenter and the hen when assessing the behavioural response of the hen to humans. In a study of withdrawal behaviour, Hemsworth et al. (1993) found that hens displayed much greater avoidance of a human standing at 30 cm distance compared with a 60 cm distance. Previous handling studies have not quantified the distance between the experimenter and the birds. For example, several studies have described visual human contact as the experimenter standing in front of the cage or walking past the cages (Jones, 1993, Barnett et al., 1994, Hemsworth et al., 1994b), but none has stated the actual distance from the cage front to where these activities were occurring. Thus, an examination of the effect of the proximity of the experimenter whilst imposing the ‘handling’ treatments was incorporated into this study.

In addition, due to the impact that early human contact can have on fear of humans later in life (Hughes and Black, 1976, Jones and Waddington, 1992, Boissy, 1995, Gross and Siegal, 1982), a rearing treatment was also included in the experiment. This component of the experiment was designed to explore how early contact with humans can influence the bird's adaptation and response to human contact at a later age. Thus, the aims of the current experiment are: (i) to examine the effects of positive and negative human handling in adulthood on the long-term behaviour, stress physiology and productivity of laying hens; (ii) to examine the effects of human handling in adulthood at varying proximities on the long-term behaviour, stress physiology and productivity of laying hens, and (iii) to examine the effects of additional human contact during rearing on the long-term behaviour, stress physiology and productivity of laying hens

3.2 Materials and Methods

3.2.1 Research Subjects

288 day-old female Hy-line White chicks were hatched on 2nd October 2006 at the poultry research facility at the Wooster campus of the Ohio State University. The chicks were wing-banded for identification purposes and raised as a single large group until two weeks of age. The chicks were housed during this time in a large indoor pen with a wood shaving substrate, and provided with *ad libitum* feed, water and heat.

At two weeks of age the chicks were individually weighed and transported to the growing facility. The growing facility consisted of two adjacent rooms (Growing Room 1 and Growing Room 2), each fitted with one row of large cages that were suitable for housing young birds in groups. The two rooms contained the same cage setup, but each was a reverse image of the other (see Figure 4). The birds were divided evenly between the two rooms to standardise their proximity to the doorway. Because of the possibility that increased human presence near the doorway may create variation in the amount of human contact that the birds received, all birds were housed on the side of the row that faced the doorway. By dividing the birds evenly between the two rooms, all birds could be housed in cages that were close to the door. It was recognised that this non-randomised design could possibly confound the results if there were differences between the front and back of the room that affected bird behaviour other than the proximity to the door, however this cage setup was necessary to minimise the human contact that the Minimal Contact birds received. The birds were housed on the second tier of the cage system. The chicks were raised from 2 to 16 wks of age in these group cages. Between the ages of 5 to 16 wks the birds received human handling treatments (described below).

The birds were transported to the laying facility at 16 wks of age, prior to reaching sexual maturity. The laying facility was located in a separate building to the growing facility, but again consisted of two adjacent rooms (Rooms 101 and 105) containing cage systems. Each cage system consisted of four rows of cages. Each row was 40 cages long, two tiers high, and had cages on either side, resulting in 160 cages per row (see Figure 5).

Each pullet was identified via wing band number, weighed and randomly allocated to a cage in one of the laying rooms. Each bird was individually housed to facilitate the measurement of egg production and behavioural variables during testing. All birds were housed in the lower tier of each row facing the door. This facilitated the ease with which the rows could be visually separated from each other, preventing birds from observing the treatments being applied to the neighbouring rows. Black plastic was draped over the back half of each row to prevent birds seeing through the cages to the row behind them, or directly ahead of them (see Figure 5.). Two cages on the end of each row were left empty, and the remaining 36 cages were divided into four blocks of nine cages. The blocks were labelled A through to D, with A being the being at the end of the row closes to the door. The human contact treatments (described below) were applied to each block.

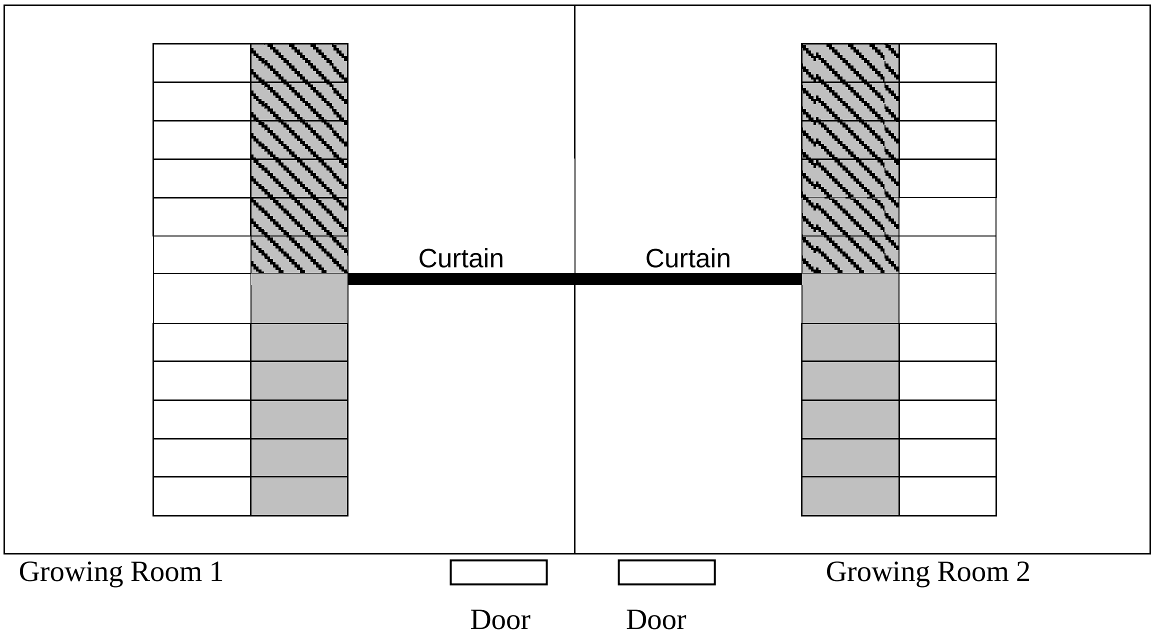


Figure 4. Diagram of cage and curtain setup in the two adjacent rooms used for rearing the pullets in Experiment 3. The shaded boxes depict cages containing the Additional Contact pullets. The shaded and hatched boxes depict cages containing the Minimal Contact pullets

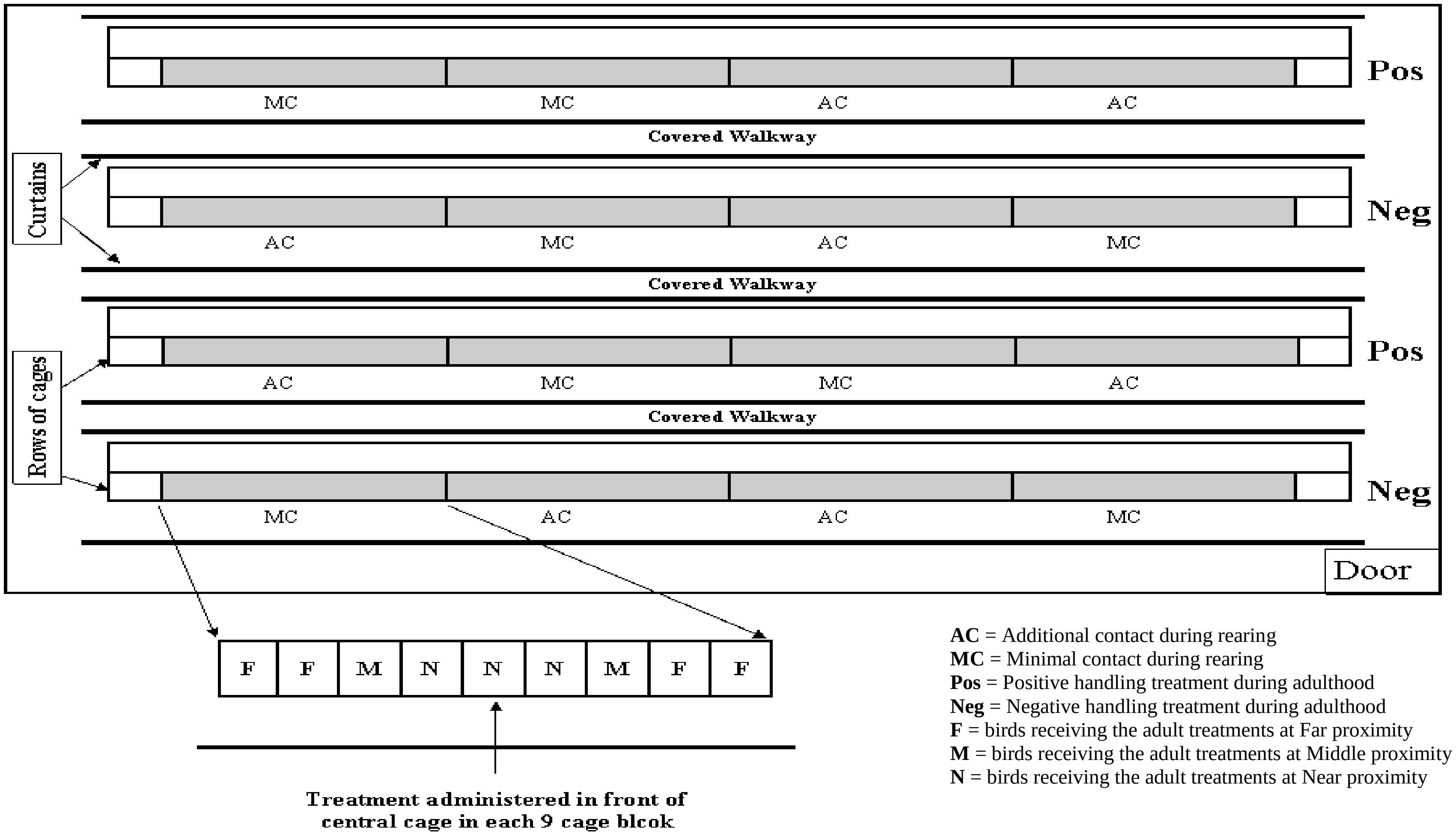


Figure 5 Cage setup in Room 101 in Experiment 2, depicting four rows of cages. The shaded boxes depict cages with birds in them. The heavy black lines depict the curtains suspended between the rows, creating the covered walkways. Room 105 has exactly the same setup, but in the reverse image

One bird was removed early in the study when it was identified as male, reducing the sample size to 287. The photoperiod was increased steadily from 10 h to 16 h during the first five weeks of the experiment in the laying facility, and the average light levels at bird height ranged from 1- 6.3 lux. Food was dispensed manually every second day, and a stick was dragged through the feed trough daily to distribute the mash evenly along the trough. Eggs were collected daily by hand. This was the only human contact that the birds received, other than the human contact treatments. The hens were provided with *ad libitum* food and water, and were transferred to a laying diet once the first egg was laid.

Room 101 had a shallow manure pit with manure belts whilst Room 105 had a deep pit. The manure belts were cleaned by pressing a switch from outside of the room and thus Room 101 did not require any additional human contact to remove manure. The manure in Room 105 simply accumulated in the deep pit until the experiment had concluded. The deep pit had a fan in the wall furthest from the door, at the end of the first row of cages. This fan let in additional light, which could be seen by the birds through the bottom of their cages. This fan and the method of manure removal were the only apparent differences between the two rooms.

The birds were individually housed in the laying cages for 20 wks, during which time they received handling treatments. At the completion of the experiment they were used for further experimental work conducted by the Ohio State University.

3.2.2 Rearing Handling Treatments

The birds were housed in the growing facility from 2-16 wks of age. At four weeks of age a curtain was hung from the middle of the ceiling in both rearing rooms, visually dividing each room in half. The curtain hung in a manner that visually separated the twelve cages into two groups of six. The six cages closest to the door in each room received additional human contact (AC) during rearing, whilst the six cages furthest from the door and behind the curtain received minimal human contact (MC). The chicks were inspected daily, and feed was manually distributed every second day. This was the only human contact that the chicks received other than the human contact treatments.

Each cage in the Additional Contact group received visual contact with a stationary human for two separate one-minute periods every weekday morning, beginning at five wks of age. This allowed the chicks one week to adjust to the presence of the curtain. During treatments, the researcher slowly entered the room holding a list of random cage-orders and moved to a distance of 1 m from the first cage on the list. The researcher then stood quietly watching the birds for one minute before moving slowly to the next cage on the list to repeat the treatment. This procedure was repeated until all cages had received the treatment twice, with the researcher spending a total of twelve minutes per day with the Additional Contact group. Care was taken not to disturb the curtain or make any noise that would disturb the birds in the Minimal Contact group. The researcher then left the room and repeated the procedure in the second room. The rearing handling treatments were imposed every week day for nine weeks, between 5 and 16 wks of age.

3.2.3 Handling Treatments

The birds were housed in the laying facility from 16-38 wks of age. The laying facility contained two adjacent laying rooms (Rooms 101 and 105), and each room contained four rows of cages. The birds were housed in the lower tier (Tier 1) only. This meant that the manure fell directly into the manure pits below the cages. If the birds were housed on the second tier then the manure would fall onto the protective flaps over Tier 1, and would require weekly cleaning. Also, the second tier was directly at face height and so the birds would receive human contact at very close proximity to the researcher's face during treatments. Considering the possible impact of eye contact on the fear response (Jones, 1980), and the fact that the previous study (Experiment 2) was conducted at a lower level relative to the researcher, the lower tier was considered the most appropriate location. The birds were housed on the same side of each row.

Black plastic was draped over one side of each row and suspended as a curtain along the other side, visually separating each row from the rest of the shed. This created a walkway down each aisle between the curtain and the back of the next row. A person was able to move up and down this walkway without being seen by any of the birds in the shed. Slits were made at regular intervals along the curtain to allow access from the walkway to the cages.

Each row was divided into four blocks of nine cages containing birds, with two empty cages at the end of each row. Each block was randomly assigned to a Rearing Treatment group so that all nine birds in a block were sourced from the same Rearing Treatment (either Additional Contact or Minimal Contact only).

The adult handling treatments were either Positive or Negative in nature, and all the cages in a single row received the same treatment. The plastic curtain was slit at the middle point of each nine-cage block, allowing the researcher access to the cages from the covered walkway. The researcher could enter the laying room and move along the covered walkway without being seen by any birds. This meant that when the researcher stepped through the slit in the curtain his appearance was unpredictable to the birds receiving the treatment.

Positive human contact treatments

The Positive treatment involved slow and predictable behaviour by the researcher so as to facilitate habituation by the birds. The Positive treatment involved 30 s of visual contact with a stationary human at very close proximity to the middle cage, in accordance with the findings of Experiment 2. The researcher administered the Handling treatments to these blocks in exactly the same order every week day (Block A through to D), and took care to move in a slow and predictable manner at all times to avoid startling the birds. The researcher entered the area in front of the first block of cages through the slit in the curtain and quietly stood as close to the cages as possible with both hands resting on the Tier 2 egg catcher holding a stopwatch. After 30 s the researcher would slowly leave the area, move along the covered walkway and emerge through the slit in front of the second block of cages. This process was repeated until all blocks in the row had received the Positive human contact treatment.

Negative human contact treatments

The Negative treatment was designed to entail startling and unpredictable behaviour by the researcher, and involved four different types of stimulus: an umbrella; a feather duster; a sheet of brightly coloured paper and human contact without a stimulus. The blocks in each row were visited in a random order, with the researcher moving unseen along the covered walkway between blocks. The researcher then pulled the curtain aside and stepped through the slit, administered the treatment stimulus and then left the area through the slit as quickly as possible. The four different stimuli were applied in the following manner:

Umbrella: The umbrella was pointed at the central cage of the nine-cage block and quickly open and shut, displaying a radially striped red and white pattern. Once the umbrella was closed the researcher left the aisle.

Folder: The blue plastic folder was held closed in front of the central cage as the researcher stepped through the curtain. Once in position, the researcher opened the folder to display a sheet of paper with brightly coloured patterns on it. The folder was held open for 1-2 s, after which it was closed and the researcher left the aisle.

Feather Duster: A feather duster made of dark ostrich feathers was quickly swept back and forth across the central cage four times, after which the researcher left the aisle.

Human only treatment: No stimulus object was used, the researcher simply stepped quickly through the curtain, stepped up to the central cage, placed their hands on the feed trough as they turned around and then left the aisle.

During the Negative treatments the order that each row was visited was randomised daily. Each block within arrow was visited in a random order to reduce the predictability of the treatments.

Proximity treatments

The proximity of the researcher was classified as either Near, Middle or Far. The central three cages in each block received the Near proximity treatment (n = 3 per block) and all Handling treatments, both Positive and Negative, were administered directly in front of the Near cages. The cage on either side of the three Near cages were labelled the Middle cages (n = 2 per block). The two cages on either end of each block were labelled as Far proximity cages (n = 4 per block). The cages were 30 cm in width and so the Handling treatments were administered at three different distances from the birds: the Near birds received the Handling treatments at 0 to 45 cm from the researcher; the Middle birds received the Handling treatments at 45 to 75 cm from the researcher; and the Far birds received the Handling treatments at 75 to135 cm from the researcher.

The experimental design is therefore a 2 x 2 x 3 factorial arrangement, combining the rearing treatments (Additional Contact or Minimal Contact), the Handling treatments (Positive or Negative) and the Proximity treatments (Near, Middle or Far). In addition to the Proximity and Handling treatments, all birds received some additional human contact during blood sampling at the beginning and end of the 20 wk adult treatment period. This procedure was likely to be more traumatic for the birds in the Minimal Contact treatment and may have influenced their response to the human contact treatments in adulthood, however the collection of a pre-treatment blood sample was a necessary component of the experimental design and this possible confounding effect was unavoidable. A timeline of significant events that occurred during the course of the experiment is depicted in Table 30.

3.2.4 Fear Assessment

Fear of humans was assessed using the Approaching Human Test (AHT), described in the methodology section of Chapter 1. The AHT was adapted for use in single bird cages by simply recording the presence/absence of the bird at the cage front during each stage of the AHT. The total number of AHT stages that the bird spent at the cage front during the test was summed to create the variable ‘TIME FRONT’, which could range between 0 and 4. The number of AHT stages that occurred before a bird withdrew from the cage front was also counted during each test. That is, if the bird was present at the cage front during the first two stages but then withdrew it would score a two. If the bird was not present at the cage front at the start of the test it would score a zero, even if it approached the cage front at a later stage. This resulted in the variable ‘TIME WITHDRAW’, which also ranged from 0 to 4. A higher score for either variable indicated lower fear of the researcher. The cages were tested in random order within each block, and the block order was also randomised. This random testing order was kept the same during the entire experiment to retain any similar variation in bird behaviour that occurred due to testing order.

Table 30 Timeline of significant events and the variables collected during Experiment 3

Age of birds (wks)	Event	Variable collected
0	Chicks hatched, wingbanded	
2	Chicks moved to rearing facility, weighed	Chick wt (g)
4	Curtain erected in rearing facility	
5 – 16	Rearing treatments imposed	
16	Pullets moved to laying facility	Pullet wt (g)
18	Behavioural testing and blood sampling	Time Front Wk 0 Time Withdraw Wk 0 Cort. Wk 0
18 – 38	Handling treatments imposed	
19	First egg laid, layer diet administered	% lay (ongoing) % abnormal eggs
23	Second behavioural test	Time Front Wk 5 Time Withdraw Wk 5
24	Egg shell weights assessed	% shell wt Wk 6
28	Third behavioural test	Time Front Wk 10 Time Withdraw Wk 10
30	Egg shell weights assessed	% shell wt Wk 12
33	Fourth behavioural test	Time Front Wk 15 Time Withdraw Wk 15
36	Egg shell weights assessed	% shell wt Wk 18
38	Final behavioural test and blood sampling	Time Front Wk 20 Time Withdraw Wk 20 Cort. Wk 20 Adult wt (g)

The birds were tested for fear of humans prior to the Handling treatments commencing (Week 0), and then tested at 5-weekly intervals for the following 20 weeks (Weeks 5, 10, 15, 20 of the Handling treatments). This created the following behavioural variables: ‘TIME FRONT Wk 0’, ‘TIME FRONT Wk 5’, ‘TIME FRONT Wk 10’, ‘TIME FRONT Wk 15’, ‘TIME FRONT Wk 20’, and ‘TIME WITHDRAW Wk 0’, ‘TIME WITHDRAW Wk 5’, ‘TIME WITHDRAW Wk 10’, ‘TIME WITHDRAW Wk 15’ and ‘TIME WITHDRAW Wk 20’. The same researcher acted as the human stimulus during testing on each test day, with the exception of the first half of the Week 0 tests, as the researcher was learning the testing procedure during this period.

Behavioural testing was conducted over two days between 0900 and 1200 h. Blocks A and D were tested using the AHT on the first day, and blocks B and C were tested using the AHT on the second day. This sampling order was implemented to remain consistent with the blood sampling procedure at Week 0 and Week 20, described in the next section

3.2.5 Stress Physiology Assessment

Plasma corticosterone concentration in response to visual human contact

All birds were blood sampled at the beginning and end of the Handling treatment period to assess any changes in plasma corticosterone concentration in response to the Handling treatments received. This resulted in the variables 'Cort.Wk0' and 'Cort.Wk20' respectively. All blood sampling was conducted after 1300 h to avoid the morning peak in plasma corticosterone related to egg lay.

Blood sampling was performed by two teams of three people. Each team included one bird handler, one blood sampler and one recorder. The sampling was conducted over a two-day period. Blocks A and D were sampled simultaneously on the first day, and Blocks B and C were sampled simultaneously on the second day. The blocks were sampled in this order because blood sampling a 9-cage block of birds exposed the neighbouring block to a substantial amount of visual contact with people, which included a certain degree of novel behaviour and equipment (eg. the use of headlamps). It was likely that this exposure would be a mild stressor for the neighbouring birds and would thus cause an increase in plasma corticosterone, confounding the measurement of plasma corticosterone to subsequent exposure to humans. Thus, by simultaneously sampling only two blocks in a row on a single day, the birds being sampled were not exposed to additional human contact prior to sampling. The row order and cage order within each block were randomly selected.

The amount of close human contact that the birds received prior to sampling was standardised to ensure that all birds were responding to a mild acute stressor. Otherwise the blood samples collected from the first birds would potentially reflect their basal plasma corticosterone concentrations whilst the last samples taken would reflect the birds' response to close visual contact with humans imposing blood sampling on the neighbouring birds. To standardise the human contact prior to sampling the bird handler and blood sampler walked slowly back and forth in front of the cages for three minutes. This was considered the minimum amount of time required for the birds to experience a corticosterone response to the close presence of humans (Beuving and Vonder, 1978). During the entire sampling procedure the recorder remained in the covered walkway with the equipment on a trolley.

The blood sampling procedure was quite rapid, and the majority of birds were sampled within 30-60 s. During the sampling procedure one researcher removed a bird from the randomly pre-selected cage and held her whilst a second researcher removed a blood sample from the wing vein. The blood samples were then stored on ice until all blood sampling was completed. The procedure was relatively quiet and apart from the close proximity of two people in front of the cages, it was relatively undisturbing for the hens.

After all nine cages in the block were sampled both researchers stepped into the covered walkway and returned to the end of the row. The first team to finish would then wait until the second team had finished, after which both teams would proceed to the next row in the random order. After all blocks had been sampled the blood samples were centrifuged and the plasma fraction removed and frozen. The plasma was later thawed and analysed by a third party using a competitive enzyme immunoassay (EIA) kit from IDS Ltd. (Fountain Hills, AZ, USA).

It was possible that the order in which the cages were sampled within each nine-cage block may have influenced the corticosterone response of the birds, with the birds sampled first being exposed to less human contact prior to sampling than the birds sampled last. An analysis of variance did not determine any significant differences ($P > 0.05$) in relation to the order of blood sampling.

Egg shell calcification

Stress is known to adversely affect the calcification of egg shells in the shell gland of laying hens (Draper and Lake, 1967). To assess any treatment effect on shell weight, the percentage shell weight was calculated on three occasions at six-weekly intervals (24, 30 and 36 wks of age). All eggs laid over a two day period were collected and kept for analysis. The eggs were weighed, cracked and the contents removed. The shells were then air-dried until their weight remained constant over two days. This usually took four to five days. The percentage of the original weight that comprised the dry shell weight was then calculated, giving the variable '%shell wt' for two days each at Weeks 6, 12 and 18 of treatments. The results from both days were then averaged to give one value per cage for each two-day period. This created the variables 'ShellwtWk6', 'ShellwtWk12' and 'ShellwtWk18'.

3.2.6 Production Records

Daily production records were kept for each cage, with all abnormal eggs noted. These records were then divided into the same five-week periods as the behavioural testing. The total number of eggs laid for a hen during each five-week period was summed and divided by the total possible number of eggs that could have been laid during each block (n = 35) and multiplied by 100 to obtain the percentage of possible eggs laid during each testing period. This resulted in the variables '%layWk1-5', '%layWk6-10', '%layWk11-15' and '%layWk16-20'. A high percentage of lay indicates that a bird is producing a larger number of eggs during that period.

The number of abnormal eggs laid per five-week block was counted for each hen and divided by the total number of eggs laid during that period and multiplied by 100 to obtain the percentage of abnormal eggs laid. This resulted in the variables '%AbWk1-5', '%AbWk6-10', '%AbWk11-15' and '%AbWk16-20'. Eggs were classified as abnormal if they were soft-shelled, cracked, contained shell bulges or deformities, or were double-yolked. Anecdotally, the technical staff reported that there was a high incidence of soft shelled eggs compared to other experiments in the same facilities. However, due to the unavailability of a matching production data set, a statistical comparison could not be made.

The bodyweight of the hens was assessed on three occasions. The birds were weighed as they were placed into the growing facility as young chicks (Chick Wt), again when they were placed in the laying facility as pullets at 16 wks of age (Pullet Wt), and finally, at the conclusion of the experiment at the age of 38 wks (Adult Wt).

3.2.7 Statistical Analysis

Differences between growing rooms (Growing Room 1 and Growing Room 2) and laying rooms (Rooms 101 and 105) for each of the variables were tested using an independent samples t-test. Of the variables affected by the main effects, only one was significantly different between growing rooms (TIME FRONT Wk 15), and two were significantly different between laying rooms (Adult Wt, % lay Wk 6-10). The small number of variables that were significantly different between rooms was not considered sufficient to impact the analysis and were thus ignored.

The corticosterone data, % lay data, behavioural data and % abnormal eggs data were not normally distributed, and several corrections were made to improve the normality of the distributions. The corticosterone data were transformed using a $\log_{10}$ transformation, which reduced the skewness and kurtosis of the data. The extreme outliers were removed from the % lay data, with a maximum of 5 outliers removed from each five-week block. This substantially reduced the skewness and kurtosis of the % lay data.

The behavioural data were highly skewed due to the large number of birds avoiding the researcher during the AHT and thus scoring a zero. The normality of the data could not be corrected, and a logistic regression analysis was considered appropriate for analysing these data. The behavioural data were recoded into a dichotomous data set in the following manner: the 'TIME FRONT' data were recoded as 1 = bird entered the front of the cage during the AHT, 0 = bird did not enter the front of the cage during the AHT. The 'TIME WITHDRAW' data were recoded as 1 = bird was at the cage front during the first stage of the AHT, 0 = bird was not at the cage front during the first stage of the AHT. A logistic regression analysis was then performed with the recoded data. If a treatment was included in the final logistic regression model for each test day then it was considered to contribute significantly to the prediction of bird behaviour. Thus, inclusion in the final model indicated a significant treatment effect.

The % abnormal eggs data were highly skewed due to the large number of zeros, as very few birds were laying abnormal eggs. The % abnormal data were also recoded into a dichotomous data set, where 1 = the hen laid an abnormal egg during a particular five-week block and 0 = the hen did not lay any abnormal eggs during that five-week period. A logistic regression analysis was performed in the same manner as for the behavioural data.

A repeated measures analysis was used to determine any treatment effects on body weight, $\log_{10}$ plasma corticosterone concentrations, % lay and % shell weight per egg. A covariate was included in the analysis if the pre-treatment measurement of a variable was significantly correlated with the same variable measured during the remainder of the experiment. Partial correlations that controlled for the each treatment were used to determine that the % lay (%layWk1-5) and the % shell weight (ShellwtWk6) were significantly correlated with the same variables, and were thus used as covariates in the repeated measures analysis.

3.3 Results

3.3.1 Bird behaviour

The full logistic regression model was compared to the constant-only model for each test day (Table 31). The full model was significantly ($P < 0.05$) different to the constant-only model on every occasion except for the Week 0 test for the variable 'TIME FRONT'. This indicates that the treatments significantly contributed to the predictive capabilities of the model, and can be used to distinguish between birds that approached the cage front during the AHT and birds that did not.

Table 31 The accuracy of the logistic regression model on each test day during Experiment 3 is summarised below, presenting the test of the full model against the constant-only model (Likelihood ratio), the amount of variation explained by the model (R^2), and the overall percentage of birds at the cage front that were correctly predicted by the model (NA = not applicable)

	Likelihood ratio	p	Nagelkerke R^2	Overall % predicted correctly
TIME FRONT Wk0	-1.75	.19	.00	NA
TIME FRONT Wk5	7.79	.02	.04	73.2
TIME FRONT Wk10	12.66	.01	.06	59.6
TIME FRONT Wk15	22.22	.00	.10	57.8
TIME FRONT Wk20	15.75	.00	.07	60.3
TIME WITHDRAW Wk0	4.09	.04	NA	NA
TIME WITHDRAW Wk5	4.04	.04	.02	79.1
TIME WITHDRAW Wk10	15.17	.00	.07	68.3
TIME WITHDRAW Wk15	14.11	.01	.07	64.5
TIME WITHDRAW Wk20	9.24	.01	.04	67.9

At Week 0, the time at which the handling treatments commenced, the birds were 18 wks of age.

The variation in bird behaviour that is accounted for is quite small, with the logistic regression model explaining 10 % or less of the variation in bird behaviour (Nagelkerke R^2 0.02 - 0.10) for every test day. Thus, whilst the treatment variables contribute to the prediction of whether the bird is at the cage front during the AHT, the strength of the association is small. This is reflected by the reduction in the percentage of birds correctly predicted at the cage front through the course of the experiment; 100% of birds that were not at the cage front were correctly predicted on each test day, while 0% of birds that were at the cage front were correctly predicted. The overall percentage that was correctly predicted decreases with time because the number of birds at the cage front increased with time. As none of these birds were correctly predicted, the accuracy of the model decreased.

The parameter estimates (B) and effect sizes for the logistic regression analysis for each test day are presented in Table 32 and Table 33. Table 32 depicts the main treatment effects, and Table 33 depicts the first order (one-way) interactions. Both tables present the proportion of birds that approached the cage front during the AHT for each treatment on each test day. A lower proportion indicates greater avoidance of humans.

It should be noted that there were no birds from the Near treatment group that were at the cage front during the first stage of the AHT for the pre-treatment tests. That is, all birds in the Near treatment group scored a zero for the variable TIME WITHDRAW Wk0. This problem is classified as a quasi-complete separation, and means that an estimate of the B coefficient cannot exist for this variable (Allison, 2004). The likelihood ratio reported in Table 31 is still valid, however the statistics for the behaviour variable TIME WITHDRAW Wk0 are not presented for the analysis of Proximity effects in Table 32 and Table 33 (denoted by NA: not applicable).

The Rearing treatments influenced the avoidance behaviour of the hens, with birds from the Additional Contact group consistently displaying less avoidance behaviour than birds from the Minimal Contact group. The Proximity treatments also influenced avoidance behaviour, with birds in the Far proximity group consistently displaying greater avoidance behaviour than birds in the Middle or Near proximity groups. There was little difference between the Handling treatments.

First order interactions occurred between the Rearing treatments and the adult handling treatments for both behavioural variables. These interactions are depicted in Table 33. In general, birds that had received Additional Contact during rearing displayed the greatest avoidance response to the Positive Handling treatment, whilst the Minimal Contact birds displayed the greatest avoidance response to the Negative Handling treatment. Both the Additional Contact and Minimal Contact groups displayed greater avoidance of humans for the Far proximity compared to the Near or Middle proximities, however the degree of avoidance was greater for the Minimal Contact group. Second order interactions occurred between the Rearing Treatments x Handling Treatments x Proximity. These data are difficult to interpret, and will not be discussed here.

3.3.2 Corticosterone concentration

There was a trend for birds receiving the Positive Handling treatment to have lower plasma corticosterone responses than those receiving the Negative Handling treatment ($F = 3.27$, $P = 0.07$) (Table 34). Plasma corticosterone concentration increased significantly over the course of the experiment ($F = 12.38$, $P = 0.00$) (Table 35).

Table 32 The proportion of birds that either approached the cage front at any point during the AHT ('TIME FRONT'), or were at the cage front during the first stage of the AHT ('TIME WITHDRAW') is presented. A high value indicates low avoidance of the researcher. B coefficients, Wald statistics and their corresponding p-values, and the (Exp)B are presented. Significant Wald values (P < 0.05) indicate that the treatment model. The (Exp)B values indicate the change in likelihood of a bird being at the cagefront for every one unit change in the predictor significantly contributed to the final regression

	Rearing Treatment						Handling Treatment						Proximity Treatment						
	AC	MC	B	Wald	p	(Exp)B	Pos	Neg	B	Wald	p	(Exp)B	Near	Middle	Far	B	Wald	p	(Exp)B
TIME FRONT Wk0	0.07	0.03			NS		0.06	0.05			NS		0.05	0.06	0.05			NS	
TIME FRONT Wk5	0.32	0.22			NS		0.27	0.26			NS		0.28	0.40	0.19	-0.41	**5.46**	**0.02**	0.67
TIME FRONT Wk10	0.48	0.33	1.89	**8.03**	**0.01**	6.64	0.39	0.42			NS		0.42	0.44	0.37			NS	
TIME FRONT Wk15	0.42	0.38	0.99	**7.54**	**0.01**	2.68	0.44	0.40	0.78	**4.70**	**0.03**	2.18	0.47	0.46	0.37	-0.50	**9.24**	**0.00**	0.61
TIME FRONT Wk20	0.48	0.31	0.80	**7.04**	**0.02**	2.22	0.40	0.40			NS		0.42	0.48	0.34	-0.38	**5.18**	**0.02**	0.69
TIME WITHDRAW Wk0	0.03	0.01			NS		0.04	0.01			NS		0.00	0.06	0.02			NA	
TIME WITHDRAW Wk5	0.26	0.16	0.59	**3.95**	**0.05**	1.80	0.26	0.16			NS		0.22	0.32	0.15			NS	
TIME WITHDRAW Wk10	0.39	0.24	2.26	**11.45**	**0.00**	9.55	0.29	0.35			NS		0.35	0.35	0.27			NS	
TIME WITHDRAW Wk15	0.41	0.30	0.76	**6.21**	**0.01**	2.13	0.36	0.35			NS		0.40	0.41	0.30	-0.43	**6.47**	**0.01**	0.65
TIME WITHDRAW Wk20	0.38	0.26			NS		0.38	0.26			NS		0.34	0.36	0.28	-0.34	**1.62**	**0.04**	0.66

Wald χ^2 (df = 1, n = 287), At Week 0, the time at which the handling treatments commenced, the birds were 18 wks of age.

Table 33 Treatment interactions for the proportion of birds that either approached the cage front at any point during the AHT ('TIME FRONT'), or were at the cage front during the first stage of the AHT ('TIME WITHDRAW'). Interactions presented are the Rearing treatment x Handling treatment, and Rearing treatment x Proximity treatment. B coefficients, Wald statistics, p-values and (Exp)B values are presented from the logistic regression analysis

	AC		MC						AC			MC						
	Pos	Neg	Pos	Neg	B	Wald	p	(Exp)B	Near	Middle	Far	Near	Middle	Far	B	Wald	p	(Exp)B
TIME FRONT Wk0	.07	.07	.04	.03			NS		.08	.09	.05	.02	.03	.05			NS	
TIME FRONT Wk5	.32	.32	.22	.21			NS		.31	.41	.28	.28	.39	.11	0.28	**5.03**	**.03**	1.33
TIME FRONT Wk10	.44	.51	.34	.31	-2.11	**5.40**	**.02**	.12	.50	.50	.45	.33	.39	.30			NS	
TIME FRONT Wk15	.42	.51	.46	.29	-3.52	**15.61**	**.00**	.03	.42	.51	.47	.52	.39	.27			NS	
TIME FRONT Wk20	.46	.50	.34	.29	-1.80	**5.53**	**.02**	.17	.42	.62	.45	.42	.32	.23			NS	
TIME WITHDRAW Wk0	.06	.01	.03	0			NS		0	.09	.03	0	.03	.02			NA	
TIME WITHDRAW Wk5	.28	.24	.18	.14			NS		.29	.34	.19	.15	.29	.11			NS	
TIME WITHDRAW Wk10	.33	.24	.44	.25	-2.42	**6.67**	**.01**	.09	.44	.41	.34	.27	.29	.20	-0.65	**5.19**	**.02**	.52
TIME WITHDRAW Wk15	.35	.47	.37	.24	-2.16	**7.16**	**.01**	.12	.37	.50	.39	.42	.32	.20			NS	
TIME WITHDRAW Wk20	.42	.35	.27	.25			NS		.33	.44	.39	.35	.29	.17	0.33	**4.24**	**.01**	.71

Wald χ^2 (df = 1, n = 287). At Week 0, the time at which the handling treatments commenced, the birds were 18 wks of age.

3.3.3 Production parameters

The results of the repeated measures analysis on production parameters are depicted in Table 34 and Table 35. Table 34 presents the differences between treatments, whilst Table 35 presents the differences that occurred over time. As expected, body weight increased significantly (F = 1983.39, P = 0.00) during the course of the experiment as the birds matured. There was also a significant effect of 'Proximity' on body weight (F = 3.21, P = 0.04), with birds in the 'Near' treatment weighing significantly less than those in the 'Middle' treatment group (P < 0.01). There was a non-significant interaction between Time x Proximity (F = 2.49, P = 0.09), with birds in the Middle group tending to gain more weight than birds in the Near or Far groups.

The rate of egg production (% lay) increased significantly (F = 8.06, P = 0.01) with time as the birds matured. There was a significant effect of the Handling treatments (F = 5.47, P = 0.02), with birds in the Positive treatment group laying a higher percentage of eggs than those in the Negative treatment group. A second-order (three-way) interaction occurred between the Rearing Treatment x Handling Treatment x Time (F = 4.48, P = 0.04). During Weeks 6 – 10 there was no significant (P > 0.05) difference between the Positive and Negative treatments groups in the number of eggs laid. During Weeks 11-15, all birds in the Positive handling treatment laid significantly (P < 0.05) more eggs than the birds in the Negative handling treatment. However, during Weeks 16-20, only those birds in the Positive handling group that had received Minimal Contact during rearing laid significantly (P < 0.05) more eggs than the birds in the Negative handling treatment. There were no other treatment effects for the egg production data.

There were no significant (P > 0.05) effects of time or treatment on the % shell weight, however there was a significant (F = 7.76, P = 0.01) interactions between the Rearing treatment x Handling treatments Birds in the Negative treatment groups laid eggs with significantly (P < 0.05) heavier egg shells than those in the Positive treatment groups, but only when they had received Additional Contact during rearing. There was no significant difference in % shell weight for Positive and Negative treatments when the birds had received Minimal Contact during rearing.

Table 34 Adjusted means (± SEM) obtained from the repeated measures analysis for treatment effects in Experiment 3. A covariate was used if the Week 0 results correlated with the results of the later tests

Dependent variable	Rearing Treatment				Handling Treatment				Proximity Treatment					
	AC	MC	F	p	Pos	Neg	F	p	Near	Middle	Far	F	p	df
Log_{10} Corticosterone (ng/ml)	0.53	0.55	0.49	0.49	0.51	0.57	3.27	0.07	0.55	0.53	0.53	0.32	0.73	2, 214
	(0.02)	(0.03)			(0.02)	(0.02)			(0.03)	(0.04)	(0.02)			
Body weight (g)	1340.9	1329.3	0.98	0.32	1328.4	1341.8	1.32	0.25	1315.9 ^{a}	1354.7 ^{b}	1334.8	**3.21**	**0.04**	2, 275
	(8.2)	(8.3)			(8.2)	(8.3)			(9.7)	(12.0)	(8.4)			
% lay (covariate included)	91.0	90.6	0.41	0.52	91.4 ^{a}	90.2 ^{b}	**5.47**	**0.02**	90.4	90.8	91.3	1.21	0.30	2, 267
	(0.38)	(0.38)			(0.38)	(0.38)			(0.45)	(0.54)	(0.38)			
% shell weight (covariate included)	9.74	9.72	0.10	0.75	9.69	9.76	1.55	0.21	9.75	9.74	9.74	0.43	0.65	2, 263
	(0.04)	(0.04)			(0.05)	(0.04)			(0.05)	(0.06)	(0.05)			

Significant differences within rows are denoted by different letters, ^{xyz} $P < 0.01$ ^{abc} $P < 0.05$.
At Week 0, the time at which the handling treatments commenced, the birds were 18 wks of age.

Table 35 Adjusted means (± SEM) for the repeated measures analysis for time effects during Experiment 3. { } denotes a covariate

Dependent variables	Week 0	Week 5	Week 10	Week 15	Week 20	F	p	df
Log $_{10}$ Corticosterone (ng/ml)	0.48 ^{x}				0.59 ^{y}	**13.02**	**0.00**	2, 214
	(0.03)				(0.02)			
Bodyweight (g)	1206.6 ^{x}				1463.6 ^{y}	**1983.39**	**0.00**	2, 275
	(5.1)				(7.7)			
% lay (covariate)		{31.14}	91.33 ^{x}	93.16 ^{y}	87.91 ^{z}	**8.06**	**0.01**	2, 267
			(0.35)	(0.30)	(0.31)			
% shell wt (covariate)		{10.21}	10.03		9.42	0.80	0.37	2, 263
			(0.03)		(0.04)			

Significant differences within rows are denoted by different letters, ^{xyz} $P < 0.05$
At Week 0, the time at which the handling treatments commenced, the birds were 18 wks of age.

The logistic regression analysis performed for the abnormal egg data revealed a trend for the full model to be different to the constant-only model for Weeks 1-5 only (Model χ^2 = 3.67, p = 0.06). During this period there was a significant (B = 0.38, P = 0.04) interaction between Rearing Treatment x Handling Treatment x Proximity for the abnormal egg data, where B = the parameter estimate used in the logistic regression model. More birds laid abnormal eggs during this period when they had received Minimal Contact during rearing, and the Handling treatments at the Middle proximity (13 % of birds for Middle, cf 0-6% for Near and Far respectively). For the Additional Contact group, the effect of the Handling treatments varied. More Far and Middle birds laid more abnormal eggs for the Positive treatment (16% and 13 % for Far and Middle respectively, 0% for Near). There was very little difference in the three proximities for the Negative treatment. The amount of variation explained by the model was small (Nagelkerke R^2 .03). There were no other treatment effects for any of the remaining five-week blocks (Weeks 6-10, Weeks 11-15 and Weeks 16-20).

3.4 Discussion

The results of the current experiment demonstrate that human handling influences the behaviour, bodyweight and egg production of laying hens.

Providing an additional 12 minutes of visual contact with a human on a daily basis during rearing reduced the fear of humans displayed by the birds in adulthood. Laying hens possess an innate fear of humans that is demonstrated by the avoidance of humans in young naïve chicks (Murphy and Wood-Gush, 1978). The chicks may view the human as a predator or simply a novel object in the environment, and thus something to be wary of (Estep and Hetts, 1992). If the chicks are repeatedly exposed to humans and during this exposure nothing aversive happens to them, the chicks will learn that humans do not pose a threat and will learn to ignore their presence. This process is called habituation, and is defined as the reduction in response to a single stimulus that occurs after repeated exposure (Broom and Johnson, 1993, Barnard, 2004).

All chicks received routine husbandry during the rearing period. However, the birds receiving an additional 12 mins of contact on a daily basis will have been given additional opportunity to habituate to humans and learn that they do not pose a threat. It is hypothesised that the Additional Contact birds were more habituated to human presence than the Minimal Contact birds at 16 wks of age, when the rearing treatments ended. However, there were no behavioural differences between the two groups when they were first tested for fear of humans at 18 wks of age (Week 0). This lack of difference in avoidance behaviour between the two groups is likely to be due to the stimulus-specific nature of habituation (Broom, 1968, Barnard, 2004). During rearing, the Additional Contact birds will have habituated to the specific type of human contact that they were receiving during rearing; that is, they became habituated to a human standing in front of the cage quietly for 2 mins a day. Any changes in this routine would initiate a change in their response (Broom, 1968). Thus, when the Additional Contact birds were exposed to the Approaching Human Test they were exposed to humans behaving in a manner that was completely novel to them. Thus, their response in the first Approaching Human Test was likely to be a response to novelty rather than a specific fear response to humans, and so both the Additional Contact and Minimal Contact birds responded in a similar manner.

Differences in avoidance behaviour between the two groups were apparent by the second behavioural test, administered after 5 wks of treatments, indicating that the rearing treatments did actually impact on the way that the birds responded to humans. The Additional Contact birds were able to habituate to the treatments and testing procedures faster than the Minimal Contact birds, as they were already used to human presence close to their cage, and thus displayed less avoidance of the approaching human. Meanwhile, the handling treatments were a completely novel experience for the Minimal Contact birds. The observed differences in behaviour between the Additional Contact group and the Minimal

Contact group were likely due to each group being at a different stage of habituation; the Minimal Contact group may simply habituate at a slower rate than the Additional Contact birds.

Bird behaviour was also influenced by the proximity treatments, with birds receiving human handling at Far proximity displaying greater avoidance behaviour than birds in the Middle or Near proximities. This is in accordance with the results of Experiment 2, in which close proximity to humans was found to reduce fear of humans in commercial laying hens. There was little, if any, main effect of the handling treatments on bird behaviour. This was an unexpected result, as the nature of human contact has previously been shown to influence the avoidance behaviour of laying hens (Hughes and Black, 1976, Jones, 1994, Jones, 1995, Jones, 1993, Jones and Faure, 1981, Zulkifli et al., 2002, Barnett et al., 1994, Reed et al., 1993, Hemsworth et al., 1994b).

Additional contact during rearing also influenced how the birds responded to the adult handling treatments. Birds that received Additional Contact during rearing displayed the greatest avoidance response to the Positive treatment, while the Minimal Contact birds displayed the greatest avoidance response to the Negative treatment. The response of the Minimal Contact birds was in the expected direction, however the response of the Additional Contact birds was not. This result may be explained by the habituation rates of the birds. The birds in the Additional Contact group were already habituated to a human presence in front of the cages, and the Positive handling treatments did not result in any further habituation for these birds. However, the Negative handling treatments may have provided a form of enrichment to the Additional Contact hens, as they were not experiencing high levels of fear of the researcher. This enrichment may have allowed them to cope with the relative novelty of the behavioural tests and thus display less avoidance behaviour, as they were also experiencing less fear of novelty. On the other hand, the adult handling treatments were the first additional human contact that the Minimal Contact group had received outside of normal husbandry routines, and the birds were likely to be afraid of all human contact. For these birds, the Positive treatments were sufficient for the birds to habituate whilst the Negative treatments were not. Thus, birds in the Positive treatment displayed less avoidance behaviour than those in the Negative treatments.

The three treatments also resulted in a second order (three-way) interaction, in which the AC+Pos birds displayed the greatest avoidance behaviour for the Near proximity, while all other treatment groups displayed the greatest avoidance for the Far proximity. These second order interactions are not easily interpreted and will not be discussed further, as their meaning is unclear. They do, however, demonstrate the complexity of the impact that human handling has on the behaviour of laying hens.

Bodyweight was influenced by handling proximity, with Middle birds weighing significantly more than Near birds. There was also a trend for Middle birds to gain more weight than the Near birds. All birds had equal access to *ad libitum* feed, so it is plausible that the Middle birds had greater feed conversion efficiency. This result is in accordance with the behavioural data, in which the Middle birds consistently displayed the least avoidance behaviour. It is therefore possible that a greater feed conversion efficiency of the Middle birds was due to lower levels of stress, as the birds were less fearful of humans. Never the less, an alternative explanation is that the Middle birds ate more. There is a substantial body of literature linking fear of humans in poultry with growth and feed conversion efficiency (Barnett et al., 1994, Barnett et al., 1992). For example, a study by Hemsworth et al. (1989) found that the avoidance behaviour of commercial broilers toward humans accounted for 28% of the variance in feed conversion efficiency. The results of the present study suggest that fear of humans could have important implications for the egg industry, in which feed is the greatest cost to the producer.

Birds in the Positive treatment laid more eggs than those in the Negative treatments. The Positive treatments were also associated with a reduction in fear of humans for birds in the Minimal Contact group. Whilst the birds in the Additional Contact group did not show a reduction in fear of humans, they were already habituated to human contact and their egg production was unlikely to be influenced by fear. These results are in the expected direction and in accordance with previous research, in which high fear of humans was associated with poorer egg production in both experimental and commercial situations (Kaltsas and Chrousos, 2007, Etches et al., 1984). The mechanism by which this occurs is likely to be due to stress hormones, resulting from fear, that interfere with the release of reproductive hormones (Draper and Lake, 1967). For example, an infusion of corticosterone will cause the ovaries to regress in laying hens, presumably by inhibiting the secretion of luteinising hormone (Draper and Lake, 1967). The fear of humans elicited in this study is unlikely to have had such a severe effect on the reproductive systems of the hens, however the results do demonstrate that there was some impact of fear on reproduction, although these data were not statistically significant.

The % shell weight of the eggs varied with Rearing treatment and Handling treatment. Birds that had received Additional Contact had the heaviest egg shells for the Negative treatments, whilst the Minimal Contact birds had the heaviest egg shells for the Positive treatments. These shell weight results coincide with the low avoidance behaviour of the hens in these treatments, and it appears that low fear of humans was associated with a greater % egg shell weight. This result may be due to the impact of adrenaline on the shell gland (Downing, 2008). Fear will elicit an acute stress response during which adrenaline is released into the blood stream. This adrenaline causes the blood to be shunted away from non-essential functions, such as egg production. This results in less blood flow to the reproductive tract and thus less calcium being transported to the shell gland to form the egg shell, causing the shell to be thinner (Hazard et al., 2005).

There was a trend for the treatments to affect the number of abnormal eggs laid during the first 5 wks of the adult treatments, when the pullets were coming into lay. A second-order (3-way) interaction occurred between the three treatments, making this result difficult to interpret. The main difference occurred between the proximity groups, with the Near birds laying very few abnormal eggs and the Middle and Far birds laying substantially more. The implications of this result are unclear.

There was a trend for birds receiving Positive handling treatments to have a lower plasma corticosterone response to close human presence than the birds receiving Negative handling treatments. This trend is in the expected direction, and implies that the birds in the Negative handling treatments experienced a greater physiological stress response to close human presence than those in the Positive handling treatments, presumably due to greater fear of humans in these birds. Previous research has shown that birds that displayed the greatest avoidance behaviour toward humans, presumably due to higher levels of fear, also had the greatest corticosterone response to handling and blood sampling (Hemsworth and Barnett, 1989, Hemsworth et al., 1994b, Barnett et al., 1994). Whilst there was only a trend for differences between treatments for the plasma corticosterone concentrations in the present study, these results do suggest that the nature of the human contact treatments may influence the birds' stress response to close human presence.

Plasma corticosterone concentrations also increased during the 20 wk period of handling treatments (Jan-June). This was an unexpected result, as the avoidance behaviour of the birds decreased with time, indicating a reduction in fear of humans. A concurrent reduction in plasma corticosterone response to handling was anticipated. Downing (2008) reported that plasma corticosterone concentration decreased with age in laying hens, however the birds used in that study were first sampled at 24 wks of age, after the onset of sexual maturity. It should be noted that the birds in the current study were sampled at 18 wks of age and again at 36 wks, during which time they reached sexual maturity and began producing eggs. In Japanese quail, the onset of sexual maturity has been associated with an increase in basal plasma corticosterone concentration, and the responsiveness of the HPA axis increased with age (Hazard et al., 2005). The increase in plasma corticosterone observed in these quail declined after reaching sexual maturity but did not drop to pre-maturity levels. This is in accordance with the study conducted with laying hens by Downing (2008). The observed increase in plasma corticosterone response to close human presence in the current study is likely to be an artefact of the maturity status of the birds. The plasma corticosterone concentrations of the hens may have peaked during sexual maturity and then began to decline, but may not have declined to the initial pre-maturity concentrations, thus appearing to increase with age.

Bodyweight and egg production both increased during the experiment as the birds reached sexual maturity. This is to be expected, and both weight gain and level of egg production was in accordance with the breed standards for the Hyline White strain (http://www.hyline.com.au/Downloads/Brochure%20(2kinds)%20Hy-line.pdf).

3.5 Summary and conclusions

The aim of this experiment was to explore the long term impacts of varying human contact on the welfare of laying hens. It was demonstrated that additional human contact during rearing and the proximity of human contact during adulthood were the most important determinants of the level of fear of the researcher displayed by laying hens during the Approaching Human Test. Additional human contact during rearing and human contact at the Near and Middle proximities were associated with a lower amount of avoidance behaviour displayed by the hens. The proximity effect is in accordance with the results of Experiment 2 (Chapter 2), in which close human proximity was found to reduce fear of humans in commercial hens when compared to more distant contact. The quality of human contact had little if any impact on fear of humans in this experiment.

Fear of humans was not correlated directly with the production of the hens. However, the same treatments that were associated with a reduction in avoidance behaviour were also associated with greater egg production, greater shell weight and greater bodyweight. These results indicate that hens experiencing high fear of humans were also experiencing a greater drain on their bodily resources, resulting in lower growth and reproductive output. These symptoms are typical of a chronic stress response, and this hypothesis is supported by the trend for highly fearful birds to have higher plasma corticosterone concentrations. It is concluded from the above study that hens submitted to handling treatments that were designed to reduce fear of humans in hens experienced less fear of humans. This resulted in the hens being able to tolerate the close proximity of humans without developing a chronic stress response, allowing the hens to have greater resource availability for growth and reproduction.

The results of this study indicate that human contact has implications for the welfare of laying hens. Fear of humans in laying hens can be manipulated by human contact during rearing and in adulthood. Furthermore, human contact treatments that were designed to reduce fear of humans were associated with a small but significant increase in egg production. This effect on productivity may be a result of reduced stress, since there was a trend for the birds to display a reduced plasma corticosterone response to close human presence. These results suggest that reducing fear of humans can improve hen welfare.

Chapter 4: Discussion of results of the three studies

4.1 Reviewing the objectives

The human-animal relationship is an important determinant of welfare in intensively farmed species (Hemsworth and Coleman, 1989a, 1998, 2000a; Breuer et al., 2000; Lensink et al., 2000a; Waiblinger et al., 2002). The quality of the relationship is largely determined by the attitudes that stockpeople have toward interacting with their animals. On farms where stockperson attitudes and consequently behaviour are poor, farm animals are more likely to be fearful of humans. Freedom from fear is one of the five basic Freedoms advocated by the UK Farm Animal Welfare Council, and these freedoms are widely recognised as important determinants of animal's welfare. In addition, fear elicits a stress response, which is undesirable from both an ethical and economical point of view.

The identification of key stockperson attitudes and behaviours that regulate the human-animal relationship in the pork and dairy industries has resulted in stockperson training programs that aim to improve this relationship (Hemsworth et al., 1994a, 2002; Coleman et al., 2000). This research has shown that by improving the attitudes and behaviour of stockpeople, fear of humans in their stock was reduced. This improvement in welfare was also associated with improvements in the reproduction of the pigs, and the milk yield of dairy cows.

Studies such as these demonstrate that the human-animal relationship has important implications for the welfare of intensively farmed pigs and dairy cattle. The egg industry is one of the largest intensive farming industries in the world, but the implications of the human-animal relationship in this industry have not yet been fully investigated. The aim of the present research was to determine the impact of the human-animal relationship on the welfare of commercial laying hens. The key areas of research in this study were:

1. To examine the existence of the human-animal relationship in the current egg industry.

2. To examine the influence of the human-animal relationship on the behaviour, physiology and productivity of laying hens.

The first area of research was addressed by the field work reported in Chapter 1, in which evidence for the relationship between stockperson behaviour and bird behaviour was found at both Australian and US egg farms. The second area of research was explored through experimental work, reported in Chapters 2 and 3, in which human behaviour was shown to influence the behaviour, physiology and production of the hens. The results of both the field work and the experimental work are summarised in the following section.

4.2 Major findings

Stockperson attitudes and behaviour

The results of the field work presented in Chapter 1 demonstrate that the attitudes and empathy of stockpeople were associated with their behaviour whilst working in the laying shed. In general, negative stockperson attitudes were associated with more noise, faster speed of movement, more time in the shed, less time spent stationary, less time at the start of the shed and less time in the ends of the aisles. The relevant negative attitudes include the insensitivity of the stockperson to the responsiveness of the hens, finding the work unpleasant, and having negative general beliefs about hens. Positive attitudes and empathy were associated with less noise and less time spent in the shed. These positive attitudes include the sensitivity of the stockperson to the responsiveness of the birds, and having positive general beliefs about hens.

Stockperson attitudes were also correlated with the number of people working in the shed, with poorer attitudes associated with larger workforces. These findings suggest that stockpeople working in large workplaces are under more pressure to conform to attitudinal norms, and it appears that the attitudinal norms on larger farms were predominantly negative.

Human behaviour and fear of humans in laying hens

Fear of humans in commercial hens, based on their avoidance of a researcher in two tests, was lower in sheds where the stockpeople spent more time standing stationary, had a faster maximum speed, and made more close cage approaches and cage contacts. Increased time spent standing stationary and close approaches are likely to reduce bird fear through habituation, but the basis of the relationship between maximum speed and fear is not obvious. More noise in the shed was associated with greater fear of humans, and this was the stockperson behaviour that was most consistently associated with fear of humans in the hens. Most of the noise that occurred in the sheds was due to the use of loud cleaning equipment, such as air hoses and leaf blowers. The hens may have become conditioned to associate humans with loud noise and so display greater avoidance when exposed to humans. Fear of humans was also correlated with the attitudes of the stockpeople, supporting the hypothesis that stockperson behaviours were mediated by attitudes, and that these behaviours were influencing the fear of humans experienced by the birds.

The proximity of visual human contact was found to influence fear of humans in laying hens, whilst the duration of this contact had no effect. In Experiment 2, daily visual contact with a human at 0 m significantly reduced fear of humans in laying hens at a commercial egg farm, whereas the same contact at 0.75 m and 1.5 m had no effect. Again, the duration of this contact had no effect on bird behaviour. In Experiment 3, birds that received visual contact with a stationary human at less than 0.75 m showed less fear of humans than birds that had received the same contact at less than 1.3 m.

Additional visual contact with humans during rearing reduced fear of humans in adulthood. In Experiment 3, an additional 12 min per day of visual contact with a human imposed from the ages of 5-16 wks resulted in a persistent reduction in fear of humans during adulthood. This may have resulted in the birds showing less avoidance of novel human contact during adulthood, as they did not perceive the presence of the researcher to be aversive. Thus, when exposed to daily human contact of a startling and unpredictable nature, the birds that had received minimal contact during rearing showed no evidence of habituation of their fear of humans, whereas the birds that had received additional contact during rearing showed further reductions in fear. This may have been due to the birds that had received additional contact perceiving human contact as rewarding rather than fear-provoking, whereas the birds that had received minimal contact were likely to perceive the human contact treatments as novel and startling. Their habituation rates would have then been delayed, and thus they would have displayed greater fear of humans during the behavioural testing.

In summary, the proximity of human contact appears to be a key determinant of fear of humans in laying hens. Visual contact at close proximities was associated with reduced avoidance behaviour in both field and experimental settings. Also, additional contact during rearing reduced the fear of humans experienced by the hens in adulthood. This enhanced their ability to cope with unexpected human contact, presumably by reducing the novelty of that contact.

Fear of humans in laying hens and the stress response

The results relating to fear and the physiological stress response of the hens are inconsistent. High fear of humans in commercial laying hens was associated with low basal corticosterone concentration in the egg albumen (Chapter 1), whereas high fear of humans in experimental birds was associated with high concentrations of plasma corticosterone in response to close human presence (Chapters 2 and 3).

It is possible that these contradictory results are due to methodological differences between the ways in which corticosterone was measured. The albumen corticosterone data collected in the field presumably represents the basal plasma concentrations of corticosterone whilst the hen is forming the egg albumen, whilst the plasma corticosterone concentrations used in the experimental work reflect the hen's acute response to a handling stressor. Thus, high fear of humans was associated with heightened plasma corticosterone response to an acute stressor in the experimental birds, and with a lower basal corticosterone concentration in the commercial birds. It should be noted that the mechanism by which corticosterone enters the egg albumen and the time course involved is not yet fully understood, and a direct relationship between plasma corticosterone concentration and albumen corticosterone concentration cannot be assumed for all circumstances. Also, as discussed in Chapter 1, the egg sample collected in the field may have been biased toward eggs that were laid by low-fear hens due to the inhibitive nature of stress on egg production. These results were then compared to the avoidance behaviour of the entire flock, possibly resulting in a misleading relationship between low corticosterone concentrations and high fear of humans on egg farms. Alternately, albumen corticosterone concentrations may have been influenced by the frequency of cleaning that occurred in the sheds. It is possible that a high rate of cleaning in the sheds may have caused the hens to be fearful of humans due to the additional noise, but the benefits of living in a clean shed were reflected in the low basal concentrations of corticosterone in the egg sample.

The physiological results from the experiments were in accordance with the literature, and demonstrated that low plasma corticosterone response to close human presence and handling was associated with less avoidance behaviour, in both the commercial (Chapter 2) and laboratory (Chapter 3) experiments. These results suggest that high fear of humans is associated with an increase in the concentration of circulating stress hormones when the hens are exposed to close human presence or gentle restraint. However, this relationship appears to be complex and influenced by many factors other than fear of humans. The difficulty involved in demonstrating this relationship outside the experimental setting is reflected by the contradictory results obtained from the field work.

Fear of humans in hens and productivity

The relationship between fear of humans and egg production was examined in the field (Chapter 1) and in Experiment 3 (Chapter 3). Again, the comparison between the field work and the experimental work produced contradictory results. In the field, fear of humans was positively correlated with egg production, indicating that more fearful birds produced more eggs. However, in the controlled laboratory environment of Experiment 3, the hens that received the positive treatments laid more eggs, indicating that less fearful birds had higher production. As for the corticosterone results discussed above, a definitive explanation cannot be provided for this discrepancy. One possibility is that the productivity of hens on commercial farms was influenced by additional factors that were not included in Experiment 3. For example, the amount of noise that occurred in a commercial shed was positively correlated with both fear and egg production. As most of the noise made in these sheds was due to motorised cleaning equipment, it is plausible that the loud cleaning increased fear of humans but also resulted in a cleaner living environment for the hens. The benefits of living in a clean shed may outweigh the negative impacts of fear on egg production.

Laying hen welfare and the human-animal relationship

Fear is a negative emotional state that may constitute suffering, to the detriment of animal welfare. As observed in the pork and dairy industries, relationships were demonstrated between stockperson attitudes, stockperson behaviour and fear of humans in hens. The relationships between human behaviour and fear of humans in hens were also shown in the experimental setting. Because fear was influenced by human behaviour in both the egg industry and in the experimental setting, human behaviour can be considered a factor that influences the welfare of commercial laying hens.

The impact of fear on the corticosterone concentration and reproductive output of the hens was not clear. On-farm, high fear of humans was associated with lower concentrations of albumen corticosterone but in the more controlled experimental setting, high fear was associated with high plasma corticosterone concentrations. The same pattern emerged for the productivity of the hens. However, the field work was an observational study, and the results cannot be used to imply causality between the variables, only association. The experimental work was conducted in a controlled environment in which key factors were manipulated to demonstrate causality. It is likely that the discrepancy in the relationship between fear and corticosterone concentration is due to additional factors on farm that were not accounted for in the experimental work, such as the cleanliness of the laying shed. In summary, the human-animal relationship was found to be an influential factor for the fear of humans experienced by laying hens, and thus was a key determinant of hen welfare.

Implications

The results of this project confirm the existence of relationships between humans and animals in the egg industry, and that this relationship has implications for the fear, and thus welfare of laying hens. An important opportunity for the egg industry that arises from this research is the development of cognitive-behavioural intervention programs for stockpeople. These training programs have already been successfully implemented in the pork and dairy industries to improve the human-animal relationship, with a resulting reduction in fear of humans in the animals occurring due to improvements in the attitudes and behaviours of the stockpeople. These results suggest that a similar cognitive-behavioural training program should be developed for stockpeople in the egg industry using the results from the current project in conjunction with recent research conducted in the European Union.

Practical recommendations

Fear of humans in laying hens was associated with the amount of noise occurring in the laying shed, the amount of close contact with stockpeople that the birds experienced, and additional contact during rearing. Thus, fear of humans can be reduced in commercial situations by altering these stockperson behaviours. Cognitive-behavioural intervention programs have been successfully implemented in the pork and dairy industries to improve the attitudes and behaviour of stockpeople while working with their animals, with associated reductions in the fear of humans displayed by their animals (Hemsworth et al., 1994a, Coleman et al., 2000, Hemsworth et al., 2002). This indicates that a cognitive-behavioural intervention program could successfully be applied to the egg industry to improve stockperson attitudes and behaviour. Thus, a recommendation arising from this research is the development and evaluation of a cognitive-behavioural intervention program on commercial egg farms. Practical recommendations cannot be made until the effectiveness of the intervention program has been determined, however the key human attitudes and behaviours that would be targeted by the program are discussed below. Basically, these cognitive-behavioural techniques involve retraining people in terms of their behaviour by firstly targeting both the beliefs that underlie the behaviour (attitude) and the behaviour in question and secondly, maintaining these changed beliefs and behaviours. These cognitive-behavioural techniques are discussed in more detail later.

A high incidence of noise was associated with greater fear of humans in commercial hens, and thus stockpeople would be encouraged to reduce the amount of noise that the hens are exposed to. The majority of noise that occurred in the sheds was due to motorised cleaning equipment, and whilst it is possible to clean the sheds using alternative methods, such as sweeping, these methods are much more labour intensive. It is unlikely that non-motorised, labour-intensive methods of cleaning will be adopted in an industry where labour shortages are rife, especially when there are no obvious financial benefits for the business. Thus, a more practical method of reducing the amount of noise that occurs in the shed would be to make stockpeople aware of the impact that noise has on the hens, and for them to reduce the amount of noise wherever possible. Reductions in noise could be made by not leaving loud equipment turned on unnecessarily, avoiding banging metal objects such as when cleaning the manure scrapers, and by placing large or heavy objects on the ground rather than throwing them.

A high incidence of visual contact with humans at close proximity was associated with reduced fear of humans in laying hens. It is impractical and unrealistic to expect stockpeople to spend time in close proximity to the birds with the sole purpose of habituating them to human contact. However, as for the method of reducing noise described above, by making the stockpeople aware of the impact of their behaviour whilst working or passing through the shed they can increase their close interactions with the birds. It would not be difficult for stockpeople to increase the number of cage approaches and cage contacts that they make. For example, they could run their hands along the feed troughs whilst walking, or stand closer to the cages than they would have otherwise. Whilst these behaviours may initially appear as an unnecessary addition to the workload of the stockpeople, the fact that cognitive-behavioural intervention programs have successfully caused behavioural change in the pork and dairy industries strongly suggests that the behaviour of stockpeople working in the egg industry could also be altered to improve hen welfare.

The relationship between additional contact during rearing and the response to the negative treatments found in Experiment 3 demonstrates that habituating birds to humans at an early age may ameliorate aversive aspects of human contact later in life. The rearing treatment utilised in Experiment 3 was simple and relatively brief, and could have lasting benefits for the hens. Thus, a further practical recommendation could be to improve the human-animal relationship early in life by exposing young chicks and pullets to regular, neutral contact with humans.

The observed relationships between stockperson attitudes and behaviour whilst working indicate the attitudes that need to be targeted to achieve change in those behaviours that regulate fear of humans in laying hens. Cognitive-behavioural intervention programs would involve presenting stockpeople with information regarding the importance of the human-animal relationship to hen welfare, and the importance of their own behaviour in relation to the human-animal relationship. Cognitive-behavioural training, which involves retraining people in terms of their behaviour by firstly targeting both the beliefs that underlie the behaviour (attitude) and the behaviour in question and secondly, maintaining these changed beliefs and behaviours (Hemsworth and Coleman, 1998), is a process of inducing behavioural change. This is a comprehensive procedure in which all of the personal and external factors that are relevant to the behavioural situation are explicitly targeted. Thus such training programs should aim to improve the stockperson's beliefs about their animals and particularly their beliefs about handling and working with their animals, by providing them with key information on their animals such as the ease with which they can and should be handled, their sensitivity to the range of negative behaviours used by stockpeople (and their sensitivity to stressors in general), and the adverse effects of these negative behaviours on their fear of humans, which in turn can have negative effects on their welfare, productivity and ease of inspection and handling. The training should also provide stockpeople with information on the positive behaviours which can be used to reduce fear in their animals. To address the behavioural aspects of the intervention, stockpeople should be shown video footage of the behaviour of stockpeople in commercial units and emphasis should be placed on those patterns, such as a high percentage of negative interactions, including moderate negative interactions, that have been shown to increase the farm animals' fear of humans. Video footage of the behavioural responses of animals to a range of stockperson behavioural patterns can also be presented to assist stockpeople in recognising and assessing fear responses in their animals. To reinforce the information targeting improvements in both beliefs and behaviours, stockpeople should be provided with written material in the form of a booklet, posters and a regular newsletter. The desired outcome of the training is to reduce the percentage of negative interactions used by stockpeople in handling their animals, that is, reduce the degree of aversiveness of their behaviour toward farm animals.

It should be noted that evidence for conformity behaviour was observed in large workplaces, where stockperson attitudes became more negative as the number of stockpeople working in a shed increased. This observation demonstrates the importance of farm management in maintaining and encouraging a positive attitude toward hens, and the importance of the human-animal relationship to hen welfare. A positive attitude by management may help improve the attitudes of stockpeople.

Whilst the relationship between fear of humans, albumen corticosterone concentration and productivity was not obvious in commercial situations, the results of Experiment 3 demonstrate that human behaviour can limit the productivity of laying hens and increase the plasma corticosterone response to an acute stressor. This result is strongly supported by the literature, and supports the hypothesis that fear of humans limits productivity in laying hens. Thus, a cognitive-behavioural intervention program that reduces fear of humans in laying hens should theoretically result in improved egg production, however further research is required in this area. It is envisaged that a cognitive-behavioural intervention program will be developed from the results of this project and in conjunction with previous research that has been conducted on this topic.

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Plain English Compendium Summary

Project Title:	Human-animal relationships in the laying hen
Poultry CRC Project No.:	03-19
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Project Overview	The human-animal relationship consists of the behavioural interactions that occur between humans and animals, and the relevant precursors and outcomes of these interactions. This study examined each of these areas within the egg industry, with particular attention to the impact of human behaviour on the fear, welfare and productivity of laying hens.
Background	The human-animal relationship consists of the behavioural interactions that occur between humans and animals, and the relevant precursors and outcomes of these interactions. One of the main precursors of human behaviour is attitude, and one of the main outcomes of human behaviour in the farming industries is the fear of humans experienced by the farm animals. Animals that are fearful of the stockpeople who care for them may experience poor welfare. Furthermore, fear through stress, may reduce productivity in farm animals. Thus, there are four facets to the human-animal relationship in farming situations: stockperson attitudes; stockperson behaviour; fear of humans in the animals, and the subsequent welfare and productivity of those animals. This project examined each of these facets within the egg industry, with particular attention to the impact of human behaviour on the fear, welfare and productivity of laying hens.
Research	*Stockperson attitudes and behaviour.* The results of the field work presented in Chapter 1 demonstrate that the attitudes and empathy of stockpeople were associated with their behaviour whilst working in the laying shed. In general, negative stockperson attitudes were associated with more noise, faster speed of movement, more time in the shed, less time spent stationary, less time at the start of the shed and less time in the ends of the aisles. The relevant negative attitudes include the insensitivity of the stockperson to the responsiveness of the hens, finding the work unpleasant, and having negative general beliefs about hens. Positive attitudes and empathy were associated with less noise and less time spent in the shed. These positive attitudes include the sensitivity of the stockperson to the responsiveness of the birds, and having positive general beliefs about hens. Stockperson attitudes were also correlated with the number of people working in the shed, with poorer attitudes associated with larger workforces. These findings suggest that stockpeople in large workplaces are under more pressure to conform to attitudinal norms, and it appears that the attitudinal norms on larger farms were predominantly negative. *Human behaviour and fear of humans.* Fear of humans in commercial hens, based on their avoidance to a researcher, was lower in sheds where

	the stockpeople spent more time standing stationary, had a faster maximum speed, and made more close cage approaches and cage contacts. Increased time spent standing stationary and close approaches are likely to reduce bird fear through habituation, but the basis of the relationship between maximum speed and fear is not obvious. More noise in the shed was associated with greater fear of humans, and this was the stockperson behaviour that was most consistently associated with fear of humans in the hens. Most of the noise that occurred in the sheds was due to the use of loud cleaning equipment, such as air hoses and leaf blowers. The hens may have become conditioned to associate humans with loud noise and so display greater avoidance when exposed to humans. Fear of humans was also correlated with the attitudes of the stockpeople, supporting the hypothesis that stockperson behaviours were mediated by attitudes, and that these behaviours were influencing the fear of humans experienced by the birds. The proximity of visual human contact was found to influence fear of humans in laying hens, whilst the duration of this contact had no effect. In an experiment, daily visual contact with a human at 0 m significantly reduced fear of humans in laying hens at a commercial egg farm, whereas the same contact at 0.75 m and 1.5 m had no effect. Again, the duration of this contact had no effect on bird behaviour. In another experiment, birds that received visual contact with a stationary human at less than 0.75 m showed less fear of humans than birds that had received the same contact at less than 1.3 m. In an experiment, an additional 12 min per day of visual contact with a human imposed from the ages of 5-16 wks resulted in a persistent reduction in fear of humans during adulthood. This resulted in the birds being better equipped to cope with novelty and human contact during adulthood, as they did not perceive the presence of the researcher to be aversive. Thus, when exposed to daily human contact of a startling and unpredictable nature, the birds that had received minimal contact during rearing showed no evidence of habituation of their fear of humans, whereas the birds that had received additional contact during rearing showed further reductions in fear. This may have been due to the birds that had received additional contact perceiving human contact as enriching rather than fear-provoking, whereas the birds that had received minimal contact were likely to perceive the human contact treatments as novel and startling. Their habituation rates would have then been delayed, and thus they would have displayed greater fear of humans during the behavioural testing. In summary, the proximity of human contact appears to be a key determinant of fear of humans in laying hens. Visual contact at close proximities was associated with reduced avoidance behaviour in both field and experimental settings. Also, additional contact during rearing reduced the fear of humans experienced by the hens in adulthood. This enhanced their ability to cope with aversive human contact, presumably by reducing the novelty of that contact. *Fear of humans and the stress response.* The results relating to fear and the physiological stress response of the hens are inconsistent. High fear of humans in commercial laying hens was associated with low basal corticosterone concentration in the egg albumen, whereas high fear of humans in experimental birds was associated with high concentrations of plasma corticosterone in response to close human presence. Nevertheless, the physiological results from the experiments were in

	accordance with the literature, and demonstrated that low plasma corticosterone response to close human presence and handling was associated with less avoidance behaviour, in both the commercial and laboratory experiments. These results suggest that high fear of humans is associated with increased concentration of circulating stress hormones when the hens are exposed to close human presence or gentle restraint. However, this relationship appears to be complex and influenced by many factors other than fear of humans. *Fear of humans and hen productivity.* The relationship between fear of humans and egg production was examined both in the field and experimentally. Again, the comparison between the field work and the experimental work produced contradictory results. In the field, fear of humans was positively correlated with egg production, indicating that more fearful birds produced more eggs. However, in the controlled experimental environment the hens that received the positive treatments laid more eggs, indicating that less fearful birds had higher production. As for the corticosterone results discussed above, a definitive explanation cannot be provided for this discrepancy. One possibility is that the productivity of hens on commercial farms was influenced by additional factors that were not included in the experiment. For example, the amount of noise that occurred in a commercial shed was positively correlated with both fear and egg production. As most of the noise made in these sheds was due to motorised cleaning equipment, it is plausible that the loud cleaning increased fear of humans but also resulted in a cleaner living environment for the hens. The benefits of living in a clean shed may outweigh the negative impacts of fear on egg production. *Summary.* Fear is a negative emotional state that may constitute suffering, to the detriment of animal welfare. As observed in the pork and dairy industries, relationships were demonstrated between stockperson attitudes, stockperson behaviour and fear of humans in hens. The relationships between human behaviour and fear of humans in hens were also shown in the experimental setting. Because fear was influenced by human behaviour in both the egg industry and in the experimental setting, human behaviour can be considered a factor that influences the welfare of commercial laying hens. The impact of fear on corticosterone concentration and egg production was not clear. On-farm, high fear of humans was associated with lower concentrations of albumen corticosterone but in the experimental setting, high fear was associated with high plasma corticosterone concentrations. The same pattern emerged for the productivity of the hens. However, the field work was an observational study, and the results cannot be used to imply causality between the variables, only associations. In contrast, the experimental work was conducted in a controlled environment in which key factors were manipulated to demonstrate causality. It is likely that the discrepancy in the relationship between fear and corticosterone concentration is due to additional factors on farm that were not accounted for in the experimental work, such as the cleanliness of the laying shed. In summary, the human-animal relationship was found to be an influential factor for the fear of humans experienced by laying hens, and thus was a key determinant of hen welfare.
Project Progress	Completed
Implications	An important consequence for the egg industry that results from this research will be the creation of cognitive-behavioural intervention

	programs for stockpeople. These training programs have been successfully implemented in the pork and dairy industries to improve the human-animal relationship. It is envisaged that a similar cognitive-behavioural training program will be developed for stockpeople in the egg industry using the results from the current project in conjunction with previous research conducted in the European Union.
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