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ABSTRACT

The preferences of animals may provide us with an indication of what is required to optimise their welfare. One method by which we can assess the preferences of animals is by use of a Y-maze apparatus, in which an animal makes a choice between two resources offered simultaneously. Although this method may seem straightforward, factors in the design of the test (methodological factors) have the potential to influence the choice of animals, leading to spurious results. This thesis, through a series of experiments, examined the effects of some fundamental factors in the design of Y-maze preference tests on the motivation and choice behaviour of laying hens. Findings from the first experiment revealed that the amount of time with a peat moss substrate impacted on hen motivation (as measured by time to choice) and potentially choice behaviour. The next experiment investigated whether this reduction in motivation was caused by the repeated interruption of dustbathing bouts. Results showed that hens did not show avoidance of an interrupted dustbathing bout in the Y-maze or show an acute physiological stress response (as assessed by measurement of plasma corticosteroids) following interrupted dustbathing bouts. To investigate the possibility that hens were unable to learn the association between Y-maze arm (location) and dustbathing bout interruption (reward), a subsequent experiment examined the learning ability of hens in a Y-maze task, where hens had to choose between an empty or peat-filled dustbath without seeing the contents of each prior to making a choice. Results revealed hens could learn the association between location and reward. A final experiment examined the effects of interval of testing and quality of substrate (type of dustbathing substrate). Results revealed neither the interval of testing nor the quality of resource provided in a Y-maze test impacted on motivation (as measured by time to choice) or choice behaviour, although further research is warranted. Results from this research have implications for the design of Y-maze preference tests in the future. This research identified some fundamental factors that need to be recognised to ensure the rigour of preference test methodology, which is necessary if we are to be assured that the results obtained from preference tests are reflective of the animals’ true preferences, which have implications for their welfare.
DECLARATION

This is to certify that

i. the thesis comprises only my original work towards the PhD,

ii. due acknowledgement has been made in the text to all other material used,

iii. the thesis is less than 100,000 words in length, exclusive of tables, maps, bibliographies

Sonja Laine.
First and foremost, I wish to thank my supervisors; Paul Hemsworth, Greg Cronin and Carol Petherick. With three supervisors in three states, I think I had a somewhat unique experience, however, by use of e-mails and teleconference meetings, I think we worked it out really well. I particularly would like to thank Paul for not only for being my primary supervisor but also for his enthusiastic influence, which helped encourage me to commence a PhD following on from my Honours research project. Greg, your expertise in hen behaviour was always helpful and I am grateful for your support with setting up the first experiment at Werribee. And Carol, your comments and knowledge of this area, relating from your earlier research, was always so valuable. All in all, I always felt that I had support and I thank you all for your time, guidance and remarks.

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CHAPTER 1

LITERATURE REVIEW

1.1 INTRODUCTION AND RATIONALE FOR THE PROJECT

Animal welfare and its assessment is a contentious issue. Although there are a number of animal welfare concepts or philosophies in the scientific literature, the assessment of animal welfare is accomplished either by examining the biological functioning of an animal or assessing an animals’ preferences. Investigating the biological functioning of an animal encompasses measurement of physiological, behavioural, health and fitness variables, making the assumption that an animal unable to cope in a situation will show biological dysfunction and this will result in poor welfare. Examining the preferences of animals enables scientists to evaluate the emotional (or subjective) state of an animal, making the assumption that the preferences of animals result from emotions (or feelings) which have evolved to motivate behaviour so that an animal will avoid harmful or aversive stimuli and select resources or environments which will optimise fitness (e.g. growth, reproduction and survival).

Animal preferences can be assessed using a number of methods and have been advocated on their practicality and consumer (or lay person) appeal (e.g. Fisher and Hogan, 2003). However, factors within the design of preference test methodology have the potential to alter the motivation of animals and thus impact on choice behaviour, leading to spurious results. Although this concern has been voiced by a number of authors (e.g. Fraser and Matthews, 1997; Mason et al., 1998), very little research has been conducted specifically examining preference test methodology. If scientists are to assess the preferences of animals, it must be ascertained that the methodology utilised is rigorous and reliable.

This PhD project aimed to examine some fundamental aspects of Y-maze preference test design and the effect these have on the motivation and thus the choice behaviour of the laying hen (Gallus gallus domesticus). The laying hen was chosen for two main reasons. Firstly, the welfare of laying hens, particularly those housed in
intensive indoor systems, is at the centre of much welfare debate. Therefore, research into improving welfare assessment methodology for this species will be beneficial for the future assessment of hen welfare. Secondly, previous research has shown that laying hens are capable of making choices in a T- or Y-maze apparatus (e.g. Dawkins 1977a; Petherick et al., 1990a, 1993; Bradshaw, 1991; Abeyesinghe et al., 2009). Therefore, findings from this PhD project may have important implications for the design of laying hen preference test methodology in the future and the principles investigated also have the potential to extend to preference testing methodology of other species.

1.2 ANIMAL WELFARE AND ITS ASSESSMENT

1.2.1 The history of animal welfare science

In the post-World War II period of the mid 1950’s, the British economy and population were expanding. As a result, the labour force was moving from agricultural industries to other industries where workers received better wages (Perry, 1983). This therefore meant that farmers were required to satisfy the consumers’ demands for cheap, safe food with less labour (Hodges, 2003). This dilemma ultimately led to the agricultural development of intensive farming systems (e.g. conventional cages for laying hens and intensive indoor housing for pigs), which allowed increased productivity and lowered production costs while keeping a high level of food quality. These systems allowed farmers to produce sufficient food supplies with limited labour at a reasonable price for the consumer.

During this time, most of the public remained unaware or unconcerned as to how farm animals were being housed and managed. This was changed in 1964 with the release of the book ‘Animal Machines’ by Ruth Harrison (Harrison, 1964). ‘Animal Machines’ informed the general public as to how intensively farmed animals were being housed and managed, including numerous photographs taken at commercial farms. The public outcry that followed from this book initiated scientific research into the welfare of livestock.

The first major result from the public outcry about farm animal welfare was the establishment of the Brambell Committee. This committee was set up by the British Government within weeks of the publication of ‘Animal Machines’ (Albright, 1998) and aimed to “examine the conditions in which livestock are kept under systems of
intensive husbandry and to advise whether standards ought to be set in the interests of their welfare, and if so what they should be” (paragraph 1, Brambell et al., 1965). The committee was comprised of scientists including veterinary and agricultural professionals and headed by Professor Brambell. The Brambell Report, which was presented to the British Government, essentially “launched animal welfare as a formal scientific discipline” (Mench, 1998).

1.2.2 Definition of animal welfare

Animal welfare may be defined as ‘the state of an individual as regards its attempts to cope with its environment’ (Broom, 1986). This means if an animal can cope well in its environment, its welfare will be good and if it cannot cope well, its welfare will be poor. The ranking of an animal’s welfare varies on a continuum scale from very good to very poor (Broom and Fraser, 2007c). When an animal fails to cope with its environment it will affect the animals’ normal functioning (i.e. challenge homeostasis). By evaluating how much has to be done by the animal in an attempt to cope with an environment or situation, the welfare of an animal may be assessed (Barnett and Hemsworth, 2003). An animal may employ a range of methods to help it cope, including behavioural and physiological responses. These responses help us assess the welfare state of an animal.

1.2.3 Animal welfare assessment

The assessment of animal welfare has been the centre of much debate and conceptual uncertainty (Sandøe et al., 2004; Barnett and Hemsworth, 2009). Currently, there is no consensus on one approach by which to assess animal welfare. Over the last three decades there has been the emergence of three broad welfare philosophies.

The “biological functioning” approach

The rationale behind this concept of welfare is that animals utilise a range of responses to assist them in coping within an environment. These responses allow animals to maintain homeostasis and therefore adapt to their environment, however, when these attempts to cope fail, the animal’s welfare will be jeopardised. Failure to
adapt to an environment, and thus failure to maintain homeostasis, may result in injury, ill health or mortality (e.g. Broom and Fraser, 1993).

The biological functioning approach to assessing welfare is based on assessing both how much an animal has to do biologically to cope with a potential threat (e.g. environment, husbandry procedure, etc.) to homeostasis and the extent to which these coping attempts are succeeding. Examining how much an animal has to do entails investigation of numerous biological responses including behavioural responses, immunological defences and physiological stress responses. The extent to which an animal is coping can be assessed by evaluation of growth, reproductive success, health, injury and disease.

The extent of coping in an environment can be assessed by examining aspects of the stress response, which includes behavioural, physiological, neuroendocrine and immunological responses. Stress is one aspect of the biological functioning of an animal that can be measured. Stress may be defined as “the biological response elicited when an individual perceives a threat to its homeostasis” (Moberg, 2000). Stress may be classified as acute or chronic, based on the duration of exposure to the perceived stressor. Acute stress results from a “relatively brief exposure to a single stressor” (Moberg, 2000), whereas chronic stress results from a “long-term, continuous” stressor (Moberg, 2000). From a welfare perspective, chronic stress can impact on the fitness of an animal and can therefore be more detrimental to an animal’s welfare compared to acute stress. It does not matter whether the perceived stressor is a genuine threat or not, it will still cause the animal to activate stress defence systems. This is why the amount of psychological stress (i.e. the stress perceived by the animal, which may or may not be real) that is experienced by an animal is likely to influence the extent of the physiological stress response (Dantzer and Mormede, 1983). An animal has four main lines of biological ‘defence’ to help cope with a potential stressor; behavioural responses, autonomic nervous system responses, neuroendocrine responses and immunological responses. Although stress is a part of everyday life for all animals, including humans, not all stress adversely affects an animals’ welfare. The welfare of an animal is affected by stress only when the animal fails to cope with the stressor.

An animal’s first line of defence for a perceived stressor is via behavioural responses. Behavioural responses are the most economical for an animal. For example, if an animal can simply move away from the perceived threat it will eliminate the stressor quickly and efficiently. Production animals, particularly those residing in
modern intensive housing systems, may be restricted in their available behavioural responses due to their confinement and lack of space. Thus, the most economical option may not be available to them.

The autonomic nervous system (ANS) is activated in conjunction with the behavioural responses (i.e. to support the behavioural responses). The ANS is part of the nervous system that controls involuntary functions that are vital to the survival of an animal, such as regulation of the heart, respiration and glands. In vertebrates, the ANS encompasses the sympathetic and parasympathetic systems that essentially work in opposition to each other (Kuenzel, 2000). The sympathetic nervous system (SNS) prepares the body for the ‘fight or flight’ response, which primes the animal to either deal with the perceived threat or flee from it. The SNS achieves this by mobilising energy reserves, increasing vital functions such as heart rate and respiration and slowing down ‘non-important’ functions (at that time), such as digestion. The parasympathetic nervous system works conversely by restoring the body to normal function. The ANS response to a stressor is a rapid but a relatively short-lived response in comparison to other responses (Moberg, 2000).

The neuroendocrine response to a perceived stressor involves activation of the hypothalamic-pituitary-adrenal (HPA) axis. As the name suggests, initiation of the response begins in the hypothalamus, where the cascade of hormonal activity begins by the release of corticotropin-releasing hormone (CRH) and vasopressin (VP) targeted at the pituitary gland (Matteri et al., 2000). The pituitary gland consequently releases adrenocorticotropic hormone (ACTH), which in turn stimulates the release of glucocorticoids from the adrenal cortex (Matteri et al., 2000). Glucocorticoids (cortisol in most mammals and corticosterone in birds and rodents) aid the maintenance of homeostasis by acting on target tissues and organs, mobilising energy stores and also playing an important role in regulating the HPA response by means of negative feedback (Fink et al., 1991). Other hormones that may be affected by the HPA include growth hormone (necessary for growth and development) and gonadotropins (luteinising hormone and follicle stimulating hormone, which are necessary for gonadal structure and function). Consequences of the chronic physiological stress response on these and other hormones ultimately result in energy being diverted away from growth and reproduction and instead preserved for homeostatic maintenance and survival (Matteri et al., 2000).
Chronic stress may also have an effect on the immune system (e.g. reviewed by Griffin, 1989; Pruett, 2003). For example, broiler chickens exposed to regular visual contact with humans from 1 to 21 days of age not only display a reduced fear response (as measured by tonic immobility), but also display a reduced stress response (as measured by heterophil to lymphocyte ratio) and improved antibody response compared to chickens which received no visual contact with humans (Zulkifli et al., 2002). Chronic stress may also lead to immunosuppression and increased disease susceptibility, as shown in numerous studies in the domestic chicken (e.g. Gross and Colmano, 1969; Gross, 1972; Mohamed and Hanson, 1980).

Consequences of the chronic stress response are often evaluated to assess the extent to which the animal is coping. Measures to assess the extent of coping can include growth rates, reproductive success, injuries and disease occurrence. For example, it has been reported that both laying hens and broilers differ in their biological response to humans based on the amount of previous handling. Both types of chickens that received regular human handling showed less fear of humans and a reduced corticosterone response to handling compared to chickens which only received minimal handling (Barnett et al., 1994; Hemsworth et al., 1994). Moreover, laying hens that received regular handling had improved egg production (a measure of reproductive success) compared to those that received minimal handling (Barnett et al., 1994). This implies that the birds that received minimal handling failed to cope with the perceived stressor (i.e. humans) as well as those birds receiving regular handling.

The “feelings based” approach

It is generally considered that vertebrates (and perhaps some higher-order invertebrates) are sentient beings, that is, they are capable of experiencing feelings. The concept of the feelings based approach to animal welfare assessment is that the welfare of an animal depends fully or partly on how the animal feels (Dawkins, 1988, 1990, 2003; Duncan and Petherick, 1991; Duncan, 1993, 2004). This approach proposes that when an animal experiences positive feelings (e.g. ‘pleasure’) the animal will have good welfare, alternatively poor welfare will result if an animal experiences negative feelings (or is ‘suffering’). Dawkins (1980) proposed that the study of animal welfare should focus on determining whether an animal is ‘suffering’. ‘Suffering’ refers to an extremely unpleasant subjective state (Dawkins, 1985a).
Although the feelings and biological functioning based approaches are often considered to differ in their concept, they are inevitably linked. This is because if an animal fails to biologically function normally (e.g. through injury, ill heath, disease, etc.), the animal will usually experience negative feelings also (e.g. pain, discomfort, nausea, etc.) (Spinka, 2006). However, it has been proposed that the feelings based approach implies that only when the animal feels ill will it’s welfare be adversely influenced, not necessarily when the animal is ill per se (Duncan, 1993).

The feelings of an animal are an aspect of its biology that has evolved to aid survival (Broom 1998, cited in Broom and Kirkden, 2004). An animal’s feelings motivate the animal’s behavioural output. If there is a deviation from homeostasis, the animal will perform behaviours to counteract the disturbance. Therefore, the feelings of an animal are dependent on the internal (primary) needs and deficiencies of the animal. For example, if an animal is dehydrated, the animal will feel ‘thirsty’ and therefore drink. If an animal has a nutrient deficiency, the animal will feel ‘hungry’ and therefore eat and so on. Thus, the feelings of an animal help an animal to survive and have optimal fitness (growth, health and reproductive success).

However, as with all welfare assessment methodologies, there has been some criticism with the feelings based approach. Bermond (1997) made the controversial claim that animals (excluding humans, apes and dolphins) are unable to experience suffering. Bermond (1997) made this claim based on the fact that animals do not have a high level of consciousness and do not have a well-developed prefrontal cortex (the part of the brain which is associated with pleasure). It is interesting to note that Bermond (1997) used the term ‘consciousness’ in a similar manner to ‘self-awareness’ (i.e. the ability of an individual to perceive its own mental life (Walker, 1988)). One may argue that it is not necessary for an animal to “perceive its own mental life” in order to suffer.

A good example of where the feelings approach to welfare seems advantageous to the biological functioning based approach is suggested by Duncan (2004). Meat animals, such as growing pigs and meat (broiler) chickens are selectively bred for fast growth rates and large appetites. The selective breeding of these animals has meant that their feelings of hunger do not match their nutrient requirements. There is no welfare concern for these animals during their growing stage, as they are able to satisfy their feeling of hunger, by ad libitum feeding, until they are slaughtered. There is, however, a welfare concern for the breeding stock of these meat animals. The breeding stock cannot satisfy their feeling of hunger, as this would result in obesity and related health
problems that would reduce the fertility of the animals. Therefore, they are restricted in their food intake. Although the animals’ nutritional needs are met, making their biological functioning suffice, the feeling of hunger still remains, making their welfare, from a feelings based approach, a concern. It has been found that broiler breeder chickens do show signs of hunger and frustration when restricted of feed (reviewed by Mench (2002)).

**The “natural behaviour” approach**

Advocates of the “natural behaviour” (or “nature of the species”) welfare assessment approach concur that, in order for an animal to have optimum welfare, it should be able to display a full repertoire of behaviours identical to that of a free living animal of the same species. For example, Rollin (1993) believes that “not only will welfare mean control of pain and suffering, it will also entail nurturing and fulfilment of the animals’ natures, what I call *telos*” (by ‘telos’, Rollin refers to behaviours or activities which are genetically intrinsic to an animal).

However, one major drawback of the natural behaviour approach is that ‘natural’ behaviour is very hard, if not impossible to define, as there are many genetic, environmental and motivational factors that influence the behaviour of any animal, whether in the wild or in captivity (Jensen and Toates, 1993; Spinka, 2006). Likewise, if this welfare approach is to be the basis of future animal welfare legislation, Appleby et al. (2004d) point out that defining or characterising a ‘natural environment’ in which farm animals are to live in order to express natural behaviour may be unattainable. For example, knowing the ‘natural environment’ of the domestic chicken may be impossible as the genetics, morphology and behaviour is different to that of its predecessor, the Red Junglefowl (Jensen, 2006).

The natural behaviour approach has been criticised by a number of authors. The main censure with this approach is that, even though the behaviours displayed by domestic animals in intensively housed systems do not match that of animals housed in extensively housed system or their wild counterparts, the intensively housed animals may not necessarily be ‘suffering’ (Beilharz and Zeeb, 1981; Dawkins, 1988). The behaviour of an animal in an intensively farmed system may vary from that of an animal housed differently. However, over the thousands of years animals have been domesticated, the genetics of domestic animals have evolved, changed and adapted
animals to their environment (Jensen, 2006). Therefore, the behaviour displayed by a domestic animal may be vastly different to that of a free living animal of the same species, though this may allow the animal to adapt to its ‘artificial’ environment. Remembering the definition of welfare from Broom (1986) (stated above), an alteration in behavioural output (as well as morphology and physiology) may, in fact, allow the animal to better cope with it’s environment and therefore enhance its welfare. Additionally some so called ‘natural’ behaviour, for example anti-predatory behaviour, observed in free living, wild animals may not be relevant for domestic animals in their artificial environment and the animal’s welfare is certainly not compromised by the deprivation of these behaviours (Dawkins, 1988).

**Combinations of approaches**

It has been suggested that for reliable results, it is best to use a number of welfare measures (Hughes, 1976; Broom, 1986; Dawkins, 1988; 1990). For example, just because an animal has an increase in heart rate does not necessarily mean its welfare is at risk. The animal may simply be in a state of arousal or may have just completed physical exercise. As stated by von Borell (2001) “A major problem of applied stress research in livestock relates to the methodology of stress assessment or to the non-specificity of the selected variable.” There are several examples in the literature. Sexual stimulation, an apparently rewarding activity, causes increased cortisol concentrations in the stallion (Colborn et al., 1991). Similarly, corticosterone concentrations increase during sexual behaviour in the male rat (Szechtmaman et al., 1974) and also during oviposition in the laying hen (Beuving and Vonder, 1981; Johnson and van Tienhoven, 1981). In addition, heart rate increases during exercise (Oritsland et al., 1977) and there is a natural diurnal rhythm of heart rate and body temperature (Khalil et al., 2004) as well as plasma corticosteroids within an animal (e.g. laying hens, (Johnson and van Tienhoven, 1981; Beuving, 1983), guinea pigs (Garris, 1979)). Therefore, the use of only one welfare assessment approach, in isolation, may provide results that are difficult to interpret.

An example of using two types of welfare assessment is from Dawkins (2003) who suggested that the welfare of an animal should be based on two key questions; “is the animal physically healthy?” and, “does the animal have what it wants?” These two questions can be answered using biological functioning based measures to answer the
first question and the feelings based approach for the second. Elston et al. (2000) used two welfare approaches in their study looking at the differences of two cage types on the welfare of laying hens. In their study, laying hens were preference tested for their choice of cage, finding there was a definite preference for one cage type over another. The experimenters then went on to assess the physiological stress response of hens housed in the two types of cages used in the preference test by taking blood samples and obtaining heterophil to lymphocyte ratios. It was also found that long-term housing in the preferred cage type resulted in a lower stress response as measured by heterophil to lymphocyte ratio. If we were to apply the two questions suggested by Dawkins (2003), the hens in the preferred cage type in the study by Elston et al. (2000) are healthy (in terms of the level of physiological stress) and do “have what they want”, as they had a distinct preference for one cage over the other. The combination of welfare assessment approaches is likely to yield more reliable and easier to interpret results.

1.3 THE LAYING HEN AND HER ENVIRONMENT

1.3.1 Introduction to the laying hen

The modern domestic chicken (Galuus gallus domesticus) was domesticated from the red junglefowl (Gallus gallus) around 8000 years ago (Oltenacu, 2005). Domestication may be defined as “the process by which a population of animals becomes adapted to living in an environment controlled by humans” (Oltenacu, 2005). The initial domestication process of red junglefowl took place in South East Asia, where the red junglefowl naturally live, and chickens were taken by humans and spread across the world (West and Zhou, 1989). As suggested by Caras (1996), the domestication of the chicken was presumably an easier process in comparison to other species, as eggs could be easily located and collected and once the chicks hatched, the imprinting process (which is the process by which “a young animal becomes attached to a ‘mother figure’…” (Manning and Dawkins, 1998)) would immediately allow the birds to form a relationship with humans.

It was likely that cock-fighting, which developed soon after domestication, had a greater influence on the global spread of the domestic chicken than its food-providing properties. Selective breeding of birds for traits associated with cockfighting also contributed to the later food-related traits. Traits selected for cockfighting birds
included large body size, lack of moulting and large comb size (an indication of androgen levels) (Wood-Gush, 1959).

Chickens were predominantly kept for cock-fighting until the mid-1800’s, after which, birds were selected by poultry breeders for exhibition (Al-Nasser et al., 2007). The breeds which were developed became the foundation of today’s modern commercial strains. The artificial selection of birds with specific production traits (e.g. increased meat or egg production) within the last 100 years has allowed for the rapid increase of the poultry industry worldwide. For example, chickens selected for egg laying improved egg production from 10 to 15 eggs per year to more than 300 (Al-Nasser et al., 2007).

As with all animals domesticated by humans, the laying hen today varies from its wild counterpart in terms of genetics, behaviour and morphology (reviewed by Jensen, 2006). For instance, the White Leghorn (a breed selected for egg production) is approximately double the size of its wild ancestor, the red junglefowl (Lindqvist et al., 2002; Kerje et al., 2003). In addition, there are behavioural differences between the red junglefowl and domesticated breeds of fowl, which suggest that the more domesticated breeds perform less ‘high energy’ behaviour (such as foraging) in comparison to the red junglefowl (Schutz et al., 2001; Schutz and Jensen, 2001).

Currently in Australia alone, there are over 19 million laying hens producing approximately 333 million dozen eggs annually (Australian Egg Corporation Limited, 2009). Worldwide, egg consumption is increasing, leading to an approximate 40% increase in production in the decade from 1995 to 2005 (Scanes, 2007). The modern laying hen is thus an important agricultural animal in terms of the sheer number of birds and production of eggs, which are a relatively cheap source of protein for human consumption.

1.3.2 Behaviour of the laying hen

Foraging and feeding

Wild and feral poultry live in a defined home range in which they know the best places to sleep, rest and feed. Although they may move up to a number of kilometres during a day, the actual movement and use of the birds’ territory will depend on factors such as the distribution of the flock, food, water and predators (Mench and Keeling,
In a semi-natural environment, free ranging red junglefowl have been shown to spend a large proportion of the day foraging, which incorporates ground pecking and scratching (Dawkins, 1989). However, more domesticated breeds of fowl have been shown to display less foraging behaviour in a semi-natural environment compared to that of less domesticated breeds of fowl (Schutz and Jensen, 2001).

The foraging behaviour of poultry consists of two parts; the appetitive component, which includes scratching and pecking, and the consummatory component, which is the actual ingestion and swallowing of the food (Appleby et al., 2004a). Hens living in a free-range environment usually scratch backwards two to three times and then step forwards to peck at the ground they have just scratched (Broom and Fraser, 2007a). Hens housed in conventional cages (see section 1.3.3) are unable to perform the appetitive component of foraging as shown by their free-ranging counterparts. It has been suggested that the manipulation of feed in the feed trough (e.g. moving feed from side to side) by hens housed in conventional cages may represent the appetitive parts of foraging behaviour (Appleby et al., 2004a).

The feeding behaviour of poultry is organised into small feeding bouts that show a diurnal rhythm (Savory, 1979). Laying hens usually feed towards the end of the day while non-layers (e.g. broiler chickens) tend to feed more in the morning. This suggests that the reproductive state of the bird has an impact on the feeding pattern (Broom and Fraser, 2007b). The formation of eggs in laying hens occurs at the end of the day, therefore, laying hens may require additional energy during this process (Savory, 1980). Feeding behaviour also seems to be socially facilitated. For example, Hughes (1971) suggested that the sight or sound of one bird feeding might influence another bird to commence feeding.

Roosting

Although the majority of the day is spent on the ground, when daylight begins to fade both feral domestic fowl and its predecessor, the red junglefowl, roost on branches in trees (Collias and Collias, 1967; Wood-Gush et al., 1978 cited in Olsson and Keeling, 2000). In the wild, feral fowl roost in the same location every night, which is the middle of the flock’s territory (McBride et al., 1969). Domestic laying hens housed in cages have also been found to be highly motivated to roost on perches at night (Olsson and Keeling, 2000; 2002). If perch access is denied, hens have shown signs
indicative of frustration, such as increased movement and time to settle (Olsson and Keeling, 2000). Perching also has a number of beneficial effects for hens. For example, hens housed with perches have increased bone strength (Hughes and Appleby 1989, 1990), improved foot condition (Barnett et al., 2005), and enhanced spatial cognitive ability (Gunnarsson et al., 2000).

Dustbathing

Dustbathing consists of a sequence of behavioural elements including scratching, bill raking, wing shaking and rubbing (for detailed description, see van Liere (1992)). Dustbathing behaviour has been observed in many avian species, however the domestic chicken (and other galliforms) may be referred to as the “dustbathing specialists” as they do not also bathe in water like other species (Simmons, 1964).

The motivation to dustbathe has been found to be dependent on an inter-play of internal and external factors. Laying hens perform, on average, one, 27-minute dustbathing bout every second day, with peak dustbathing activity around midday to early afternoon (Vestergaard, 1982; Vestergaard et al., 1990; Hogan and van Boxel, 1993). This is suggestive of an endogenous circadian rhythm of motivation. In addition, following a period of dustbathing deprivation, hens have been found to reduce the latency to dustbathe and increase the dustbathing bout duration when dustbathing is next allowed (Vestergaard, 1982), conforming to the Lorenzian ‘psychohydraulic’ model of motivation (Hogan and van Boxel, 1993). Additionally, there is evidence to suggest that dustbathing deprivation can cause an increase in plasma corticosterone concentrations, a sign of physiological stress (Vestergaard et al., 1997). This implies that dustbathing deprivation may be stressful.

Dustbathing may also be influenced by external factors. For example, lighting (Hogan and van Boxel, 1993; Duncan et al., 1998), temperature (Duncan et al., 1998) and the sight of a dusty substrate (Petherick et al., 1995) have all been found to influence dustbathing behaviour. Dustbathing may also be socially facilitated as, when hens have free access to a dustbathing substrate, they are often observed performing dustbathing behaviour in synchrony (Vestergaard, 1982). Moreover, Duncan et al. (1998) found that individually caged hens dustbathed more often when they had visual contact with a stimulus dustbathing hen compared to when the hens could only see another dustbath. However, recent research suggests that social factors may not
influence dustbathing behaviour as first thought (Olsson et al., 2002a; Lundberg and Keeling, 2003). For example, Olsson et al. (2002a) reported that when test hens received a dustbathing substrate in the presence of a stimulus hen (which was either dustbathing or performing a different behaviour), stimulus hens had no effect on the dustbathing behaviour of test hens.

The main function of dustbathing is to regulate feather lipids and thus maintain plumage and it is possible that feather condition is a causal factor for this behaviour (van Liere and Bokma, 1987). However, there has been some uncertainty concerning this theory in the domestic chicken. Although birds in the wild require feathers to be in good condition for survival (e.g. for insulation and flight) (Middleton, 1991), for domesticated poultry species, particularly those in intensive indoor housing systems, this requirement is obviously not as important. Even so, it has been found that laying hens will dustbathe in the absence of a suitable dustbathing substrate (referred to as ‘sham’ or ‘vacuum’ dustbathing) (Lindberg and Nicol, 1997; Olsson et al., 2002b). Furthermore, featherless (scaleless) domestic chicks display dustbathing behaviour (Vestergaard et al., 1999), even though it clearly serves no function in regards to plumage maintenance. This suggests that the motivation to dustbathe does not necessarily depend on feather condition.

Laying hens have been found to prefer specific substrates in which to dustbathe. Research suggests that substrates which are composed of a smaller particle size, such as sand or peat moss, are preferred dustbathing substrates compared to materials with a relatively larger particle size, such as sawdust or shavings (Petherick and Duncan, 1989; van Liere et al., 1990; Shields et al., 2004; de Jong et al., 2007). This preference is possibly due to the fact that substrates with a smaller particle size are more efficient at reaching the proximal (plumulous) part of the feathers (van Liere, 1992) and thus provide a more effective dustbath in terms of removing excess feather lipids. Furthermore, when given sawdust as a dustbathing substrate, laying hens have been found to take longer dustbathing bouts (van Liere et al., 1990) in comparison to hens given sand, again suggesting that sawdust is an inferior quality dustbathing substrate compared to those with a smaller particle size.
Social behaviour

When housed together, hens (and chickens in general) display affiliative behaviour by flocking, which has primarily evolved for protection against predators (Appleby et al., 2004b). Domestic hens in a flock form a hierarchy based on the dominance of each individual. The hierarchy is developed by birds ‘testing’ each other and is maintained by non-physical behaviour, such as threats (Williams et al., 1977; Mench and Ottinger, 1991). This hierarchy has been found to be very stable and may be maintained for a long period, up to a number of years (Schjelderup-Ebbe, 1922 cited in Mench and Keeling, 2001). Distinguishing features which may aid hens to recognise individuals and identify hierarchy status include comb size and colour and body size (Mench and Keeling, 2001). The social rank of a hen has been found to influence a number of individual bird traits when housed in conventional cages, including egg production and frequency of feeding (Cunningham and van Tienhoven, 1983). In addition, when housed in furnished cages (see section 1.3.3.2), dominant hens have been found to have priority of dustbath access over subordinate hens (Shimmura, et al., 2007).

Laying hens are able to recognise and discriminate between other individuals by visual features (Guhl and Ortman, 1953; Dawkins, 1995; D’Eath and Stone, 1999; Hauser and Huber-Eicher, 2004). Several studies have demonstrated that laying hens prefer to be closer to familiar conspecifics rather than unfamiliar, and to subordinate hens than dominant hens (Dawkins, 1982; Bradshaw, 1992; Grigor et al., 1995). However, factors such as type of assessment task (Abeyesinghe et al., 2009) and distance to the other individual (Dawkins, 1995) may influence this preference.

Social contact is presumably important for laying hens. When isolated, domestic chicks have been found to show behavioural and physiological indicators of stress, however, these stress indicators were reduced when the test chick was placed in the same environment with a conspecific (Jones and Merry, 1988). Moreover, Jones (1984) found that the fear-related responses of a chick in an open field were reduced when the conspecific was a familiar chick compared to an unfamiliar chick. In adult laying hens several behaviours have been found to be socially facilitated with many individuals performing the behaviour in synchrony. Examples include feeding (Hughes, 1971; Webster and Hurnik, 1994), preening (Webster and Hurnik, 1994) and possibly dustbathing (Duncan et al., 1998). Furthermore, Mills and Faure (1989) reported that when hens were isolated from familiar conspecifics, the isolated hen showed reduced
feeding and increased behaviours that may be related to frustration (movement and preening), implying that social isolation may cause frustration. This indicates that social contact is important for laying hens.

*Reproductive behaviour*

The behavioural patterns associated with egg laying in the laying hen are relatively consistent between birds as it is under genetic control (Appleby *et al.*, 2004c; Mench, 2009). The commencement of pre-laying behaviour is initiated by the release of oestrogen and progesterone from the follicle (Wood-Gush and Gilbert, 1973). During this pre-laying period, hens are highly motivated to find a nesting site (e.g. Duncan and Kite, 1987; Cooper and Appleby 1996; Kruschwitz *et al.*, 2007) and perform nest-searching behaviour. In the conventional cage, where no nesting site is provided (see Section 1.3.3.1), this nest-searching behaviour is represented by restless pacing (Petherick and Rushen, 1997). Once a suitable nesting site has been chosen the hen creates a nest hollow in the litter by using her feet and rotating her body (Mench, 2009). In housing systems that permit it, this nest searching and nesting behaviour in the domestic hen is similar to that observed in a natural environment (Wood-Gush, 1971 cited in Appleby *et al.*, 2004c). The complete process of egg laying, from pre-laying behaviour to oviposition, takes approximately 1-2 hours, but may be extended if no nesting site is available (Mench, 2009).

Hens housed in furnished cages and non-cage systems (see section 1.3.3) are provided with nest boxes in which to lay. When hens are not provided with a nest box or litter in which to lay, as is the case in conventional cages, hens are often observed displaying abnormal behaviour, such as stereotyped pacing (Mills and Wood-Gush, 1985; Ramos and Craig, 1988). This is indicative of long term, intense frustration (Duncan and Wood-Gush, 1972; Broom and Fraser, 2007b). In addition, research by Wood-Gush and Gilbert (1964; 1973) has shown that the motivation for pre-laying behaviour is initiated by hormonal control (progesterone and oestrogen), meaning it is an internal factor which is presumably present in all hens, irrespective of the environment in which they are housed.
1.3.3 Laying hen housing systems

In the commercial egg industry there are a number of laying hen housing systems in use. The predominant system in Australia is the conventional cage system. Figure 1.1 shows the percentage of eggs produced in each system for eggs produced in Australia. All laying hen housing systems have both advantages and disadvantages and therefore no housing system is perfect in terms of hen welfare and production costs.

![Figure 1.1 Percentage of eggs produced in different housing systems in Australia in 2008 (Australian Egg Corporation Limited, 2009).](image)

The conventional cage

The conventional (or “battery”) cage system was first introduced in Australia in the 1950’s (Cransberg and Parkinson, 2007) and is one of the most controversial housing systems in livestock production due to the confinement of, and lack of stimulation for the animals. The conventional cage is simply a metal wire cage with an exterior feed trough (which may be automated for feed delivery), an automated watering system and a sloped floor, which allows eggs to roll out to an egg collection tray. The perforations in the wire flooring allow faeces to drop through which removes waste from the cage. In modern cage systems, the faeces fall onto a conveyor-type belt beneath the cage that allows the waste to be removed from the facility.
Each cage may house any number of hens, depending on the size of the cage. The minimum space allowance currently in Australia is 450 cm$^2$ per hen, however, this is being increased to 550 cm$^2$ for all cages built after January 2001 (Primary Industries Standing Committee, 2002). Due to the low cost of production, cage eggs are the cheapest source of eggs for the consumer, which is probably why the cage system produces the highest proportion of eggs in Australia (Figure 1.1).

In addition to producing eggs cheaply, the conventional cage has other benefits. As birds are separated from their faeces, the cage system has the lowest incidence of disease and is the most hygienic system for birds, staff and eggs (Larsson et al., 1999; Michel and Huonninc, 2003). Also, as cages are kept inside large sheds, environmental factors such as temperature, light and ventilation are easily controlled. As the flock is separated into cages to form relatively small groups of birds (in contrast to the large flocks in barn or free-range systems), the occurrence of aggression and feather pecking is minimised and any outbreaks of feather pecking or cannibalism does not spread throughout the whole flock (Cransberg and Parkinson, 2007). Finally, the production of eggs by hens housed in cages has been found to be better than other systems in terms of number of eggs produced, egg quality and feed efficiency (Wegner, 1983). A summary of strengths and weaknesses for all laying hen housing systems is presented in Table 1.1.
Table 1.1    Major strengths and weaknesses of the main types of laying hen housing systems. (Cransberg and Parkinson, 2007).

<table>
<thead>
<tr>
<th>Housing type</th>
<th>Strengths</th>
<th>Weaknesses</th>
</tr>
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| Conventional Cage | - Good disease control.  
- Good automation and control of feed, water, ventilation, light and temperature.  
- Hygienic for birds, eggs and staff.  
- Cost effective.  
- Minimal aggression, feather pecking and cannibalism.  
- Easy to inspect, identify and treat any ill birds.  
- Small groups; outbreaks of feather pecking do not spread through whole flock. | - Behavioural deprivation of behaviours such as walking, scratching, nesting, dustbathing, perching and stretching.  
- Foot problems.  
- High incidence of osteoporosis and broken bones.  
- No escape from aggression, feather pecking and cannibalism if it occurs. |
| Furnished Cage | - Good disease control.  
- Good automation and control of feed, water, ventilation, light and temperature.  
- Hygienic for birds, eggs and staff.  
- Cost effective.  
- Allows expression of some behaviours, such as perching, dustbathing and nesting.  
- Increased bone and muscle strength.  
- Easy to inspect, identify and treat any ill birds.  
- Small groups; outbreaks of feather pecking do not spread through whole flock. | - Prevents expression of some behaviours, such as walking, stretching, wing flapping.  
- No escape from aggression, feather pecking and cannibalism if it occurs. |
| Barn | - Full behavioural expression possible.  
- Increased bone strength.  
- Control of feed, water, ventilation, temperature and light.  
- Relatively easy to inspect birds | - Disease and mortality higher than cage systems.  
- Large group; risk of feather pecking and cannibalism.  
- Higher production costs.  
- Presence of dust and ammonia, less hygienic than cage systems.  
- Harder to identify and treat ill birds. |
| Free Range | - Full behavioural expression possible.  
- Access to outdoors.  
- Varied diet and environment. | - Increase in disease and parasitism.  
- Increased use of chemicals.  
- Predation and fear of predation.  
- Environmental factors hard to manage.  
- High labour costs and incidence of dirty eggs, therefore higher production costs.  
- Generally small scale.  
- Decreased biosecurity (e.g. diseases from migratory wild birds).  
- Hard to inspect birds and to identify and treat ill birds.  
- Hard to manage and contain feather pecking/cannibalism outbreaks. |
The welfare concerns for the hens housed in the conventional cage are predominantly associated with the lack of space and the barren, unstimulating environment. In a study by Dawkins and Hardie (1989) it was found that the space required by hens to perform behaviours such as preening, wing stretching and flapping is more than the 450 cm² allowed by some cages. For example, the space requirement for wing flapping was found to be 860 to 1980 cm² and the space required for preening was 814 to 1270 cm² (Dawkins and Hardie, 1989). This implies that some behaviour may be restricted in conventional cages. Furthermore, certain behaviours which have been found to be highly motivated at certain times of the day, such as nesting, perching and dustbathing are also prevented and, as discussed above, the prevention of these behaviours appears to result in frustration and/or stress (e.g. Vestergaard et al., 1997; Olsson and Keeling, 2000; Broom and Fraser, 2007b) and is thus a welfare concern.

In addition to behavioural restriction, hens housed in conventional cages may also suffer from physical abnormalities. It has been found that the bones of hens housed in cages are different to those of hens housed in other systems that allow more space. Caged hens have a higher incidence of broken bones compared to those housed on floors (Meyer and Sunde, 1974). This increase in bone breakages is most likely due to the decreased bone strength of hens housed in conventional cages due to the restriction in movement and therefore exercise that exists in these cages (Meyer and Sunde, 1974; Knowles and Broom, 1990; Fleming et al., 1994; Michel and Huonnic, 2003).

The welfare of hens housed in conventional cages has become such a welfare concern that it has influenced poultry housing legislation. The European Union (EU) has agreed to phase out conventional cages for egg production by 2012 following the EU Council Directive 1999/74/EC. The judgement of where to draw the line on laying hen housing (and other welfare issues) tends to be a cultural and political issue rather than a scientific one (LayWel, 2006). Although science can inform society, a recent paper by Savory (2004) suggests that the decision to ban battery cages in Europe has arisen more from public outcry and political reasons rather than from sound scientific evidence. In Australia, one of the major supermarket chains has decided to phase out a number of cage egg products due to a declining customer demand (Collerton, 2009). Additionally, there have been several attempts to ban battery cages in Australia. In 2007, the Australian Capital Territory (ACT) unsuccessfully put forward laws banning battery cages in the ACT (Beeby, 2007). Recently, the Greens political party in
Tasmania have put forward a bill to ban battery cages in that state (Rae, 2008), following a failed attempt in 2000.

The furnished cage

While furnished cages are not yet in commercial use in Australia, these cages have been accepted by the EU as a suitable alternative to conventional cages. The furnished (or “enriched”) cage is aimed at increasing environmental complexity and adding features which permit the behaviours which hens are seemingly highly motivated to perform (e.g. nesting, perching and dustbathing), while still keeping the advantages of the cage system such as reduced disease prevalence, good cost effectiveness and enhanced environmental control. The furnished cage includes a number of pieces of ‘furniture’ including perches, nest boxes and dustbaths (Figure 1.2). Hens housed in furnished cages have greater bone and muscle strength compared with those housed in conventional cages, as the birds gain exercise from the inclusion of perches (Hughes and Appleby, 1989; Knowles and Broom, 1990; Leyendecker et al., 2005). Interestingly however, physiological stress measures such as adrenal responsiveness are seemingly not different between hens housed in furnished cages and in conventional cages (Guesdon et al., 2004).

Furnished cages also allow more room per bird in comparison to conventional cages; 750 cm² per bird (Department for Environment, Food and Rural Affairs, 2002). These cages may contain up to 60 birds per cage, depending on the size of the cage (Pickett, 2007).

Rodenberg et al. (2005) reviewed the benefits and disadvantages in terms of welfare, health and hygiene of furnished cages to other alternative (non-cage) systems. The authors conclude that furnished cages are better than alternative non-cage systems in terms of health and hygiene and the occurrence of feather pecking, however, alternative systems allow more movement and provide greater environmental complexity.
The barn system

Barn housing systems, originally developed in Europe, house flocks of birds (up to 10,000 hens) in large, deep litter floored sheds with no outdoor access. Birds have access to nest boxes and perches. This system allows birds to express a full range of behaviours and less confinement than the cage systems. Additionally, being an indoor system, the risk of predation is eliminated, hens are protected from adverse weather and biosecurity is increased in comparison to the free-range system.

However, having hens in such a large flock does increase the chance of feather pecking and cannibalism and there is a higher incidence of disease (Cransberg and Parkinson, 2007) compared to cage systems, possibly due to the birds being in close contact with faeces. Also, there can be a build up of dust and ammonia, which can affect not only the health of the birds but the health of staff as well (Larsson et al., 1999; Michel and Huonnic, 2003). In addition, there may be a higher incidence of ‘dirty’ eggs (i.e. eggs which have manure/flooring substrate adhered to them and thus
reduce quality) and therefore increased production costs in comparison to the cage systems (Cransberg and Parkinson, 2007).

The barn system only accounts for approximately 5% of the eggs produced in Australia (refer Figure 1.1). Department of Primary Industries (2009) suggests that this is due to consumers having only limited knowledge and understanding of the barn system and its benefits compared to free range and also the higher cost of barn eggs compared to eggs produced in conventional cages.

The free range system

The free range system allows birds a large indoor area containing deep litter and perches (similar to the barn system), with access to a mainly vegetation-covered outdoor area during the day (Glatz and Ru, 2004). Like the barn system, this system allows birds to display a full repertoire of behaviours, including dustbathing, foraging and roosting. Theoretically, this system has a low stocking density in comparison to other systems (1 bird per 10m² for the total indoor and outdoor area) therefore requiring significantly more space. However, research suggests that only a small proportion of hens in a free range flock make use of the outdoor area and the majority of the flock remains indoors, making the stocking density of birds in a free range system variable, with a higher stocking density indoors than outdoors (Keeling and Hughes, 1988; Weeks et al., 1994). It is possible that the ability of hens to perform ‘natural’ behaviours and freely move around in a large quantity of outdoor space also makes the free range system aesthetically appealing to the consumer and general public (Department of Primary Industries, 2009).

Although the free range system does allow hens a large amount of space and opportunity for ‘natural’ behaviours, there are a number of negatives as well. As the birds are allowed outdoor access, there is the risk of predation from animals such as foxes, cats and birds of prey (Lölinger et al., 1981 cited in Broom and Fraser, 2007b). In addition, the incidence of disease may be greater than that of a cage system (Lölinger et al., 1981 cited in Broom and Fraser, 200b) due to hens being in closer contact with their faeces and there is the risk to biosecurity (i.e. disease being transmitted from wild birds to hens). The labour required to run a free range enterprise is much greater than the cage systems due to the large space required and there may be a higher incidence of dirty eggs, again increasing production costs (Department of Primary Industries, 2009).
1.4 ANIMAL PREFERENCE TESTING

1.4.1 Introduction

Animal preference tests allow scientists to ‘ask’ an animal which resource, environment or performance of a behavioural pattern it prefers in comparison to another. Preference testing is a unique methodology as it is an objective technique to assess how an animal perceives its environment (Dawkins, 1980).

A fundamental assumption in animal preference tests is that animals are able to choose what is best for them. It is assumed that animals should have evolved to prefer an environment that optimises survival and fitness, as only animals that choose an environment that is appropriate can grow, reproduce and raise offspring. An animal’s preference is seemingly linked to its emotional experience at the time of making a choice. Presumably, animals should choose environments or resources that allow positive feelings (e.g. pleasure or comfort) and avoid environments that elicit negative feelings (e.g. fear or discomfort) (e.g. Dawkins 1980; Fraser and Matthews, 1997). Hence, it is proposed that animal preference tests are based on the feelings of animals, as it is the animals’ feelings that underpin animal choices. In addition, this technique links the feelings of animals with the biological functioning approach as animals should also select environments that help maintain homeostasis (Fraser and Matthews, 1997).

However, the ability of animals to choose what is best for them has been the subject of debate. For example, Duncan (1977) argued that domestic animals might not be able to make ‘wise’ choices due to the years of domestication and selective breeding for production traits. Unlike their wild counterparts, domestic animals rarely have to make choices that influence their fitness due to the artificial environments in which they are housed (e.g. readily available food, shelter, veterinary care, etc.). For example, cattle may choose to consume toxic plants while grazing (e.g. Pfister et al., 1988; Ralph and Olsen, 1990) and domestic cats may exhibit pica by chewing, sucking or ingesting non-nutritive objects such as wool or cotton (Bradshaw et al., 1997) which may lead to health problems such as bowel obstructions. Kare and Ficken (1963) went as far as to say that the “inherent ‘nutritional wisdom’ of domestic animals cannot be relied on in (dietary) preference tests”. In defence of this, Dawkins (1977b) suggests that animals may not always choose what is best for them, though this is not necessarily due to domestication. Dawkins (1977b) states that the artificial environment in which animals
are preference tested may influence the animals’ choice. For example, rats may choose to travel a distance through extreme cold to access a sweet novel food (e.g. cake) when a perfectly nutritious food is freely available (Cabanac and Johnson, 1983). Dawkins (1977b) suggests that this may be because in nature there is rarely an example of a food that is sweet tasting but non-nutritious.

Likewise, Fraser and Matthews (1997) point out that animals may not be able to choose what is best for them if they are exposed to potential dangers or benefits which lie outside of their sensory or affective capacity. They use the example of aquatic contaminant avoidance in fish. Fish prefer to avoid an environment containing contaminants such as copper (Giattine and Garton, 1983, cited in Fraser and Matthews, 1997), however, when exposed to other pollutants, such as phenol (a human-made chemical), they generally do not show this preference (Giattina and Garton, 1983, cited in Fraser and Matthews, 1997; Hartwell et al., 1989). This lack of preference is presumably because fish did not develop the capacity to detect these pollutants during evolution. Therefore, when asking an animal to make a choice, we must be sure that the question we are asking them to make is within their sensory capacity and is similar to that which they would make in normal life.

There are also examples of animals being able to choose what is best for them. Danbury et al. (2000) reported that lame broiler chickens chose a diet that contained carprofen (a drug which reduces pain and inflammation) whereas healthy, sound chickens did not show this preference. Thus, although there are examples of animals choosing both what is best for them and what is not, animal preference tests rely on the assumption that animals should choose what is in their best interest, or what makes them experience a positive subjective state.

Animal preference tests do have the benefit of answering ‘animal-centred’ questions without the influence of human presumptions. For example, in the Brambell report, a section stated that laying hen cage flooring should not be constructed of fine gauge wire netting of a hexagonal design (paragraph 68, Brambell et al., 1965). The advisory committee thought that this flooring was not appropriate for the hens’ feet. Instead, they advised that rectangular designed metal mesh flooring was better. Several years later Hughes and Black (1973) conducted a preference test in which laying hens were allowed free access to different floorings, including the hexagonal and rectangular designs mentioned by the Brambell report. Using the amount of time spent on each type of flooring as the measure of preference, it was found that the hens had a preference for
the hexagonal design flooring, possibly because it provided more points of contact with the hens’ feet and therefore supported the hens’ feet better than other flooring. The study by Hughes and Black (1973) illustrates the usefulness of animal preference tests and how useful this technique can be in determining what the animals themselves prefer rather than assumptions made by humans.

There are also other advantageous aspects of utilising preference test methodology. Firstly, this technique gives scientists an insight into the subjective feelings of an animal. This is important, as the welfare of an animal is dependent (at least partly) on its feelings (see section 1.2.3). Secondly, the method itself offers a practical and convenient technique where many trials can be run in quick succession. Additionally, this type of welfare assessment is likely to be more appealing and easily understood by the consumer and general public in comparison to other techniques such as the biological functioning methods (e.g. Fraser et al., 1993; Fisher and Hogan, 2003).

However, there are also a number of criticisms that have been raised concerning animal preference tests. One of the main criticisms is the effect of experience by the animal in relation to the environment on offer. For example, Beilharz and Zeeb (1981) stated “with appropriate rearing and prior experience almost any familiar environment will be preferred over other environments.” While the previous experience of an animal has been shown to have an effect in some studies (Dawkins, 1976; 1977a; Hughes, 1977; Petherick et al., 1990a), this is not always the case. For example, Dawkins (1978) showed that hens raised in small cages and hens raised in large outdoor housing (a hen house with an attached run) both preferred the same environments with respect to size and flooring type. However, to control for experience, animals used as experimental subjects in preference tests must be familiar with all resources or environments under investigation.

Another criticism of animal preference tests is that animals may not be able to choose between choices that conflict between long-term and short-term welfare (Duncan, 1978, 1992; van Rooijen, 1982). For example, as mentioned previously, broiler chickens have been bred to have such a large appetite, were they not slaughtered at a young age, they would have problems with general health and reproduction. When animals make a choice in a preference test, their choice will be based on their feelings at that point in time and this must be kept in mind when interpreting animal choices.

Animals rarely, if ever, choose one resource or environment over another 100% of the time and this has been identified as a limitation of animal preference tests and this
non-exclusive choice by animals has been suggested as making results difficult to interpret (e.g. Kirkden and Pajor, 2006). However, Duncan (1978) suggested that this might have some adaptive value for animals. For example, when animals are placed in an environment where they must make a choice, initial sampling of each option may allow the animal to gain information required to make a well-informed choice (Nicol, 1986). Furthermore, once a choice has been made, monitoring the other options in the choice test may allow the animal to detect if the less preferred option has significantly changed since the animals’ last visit (Arnold, 2005). Another explanation may be that when many tests are run consecutively an animal may experience satiety with the preferred resource and thus have time to explore the less preferred option. Other possible causes that could lead to a non-exclusive choice may be individual variation, misidentification of resources or the change of preference over time (Nicol, 1986). Therefore, it should come as no surprise that animals do not choose an option on 100% of test trials.

In summary, there are a number of criticisms in relation to animal preference tests, but these criticisms may also be associated with other welfare assessment methodologies (reviewed by Dawkins (1983a)). The main advantage that animal preference tests offer is to gain insight into animal feelings. As Dawkins (1980) states “(when) properly used and taken in the context of information from other sources, they (animal preference tests) give us the closest approach we yet have to being able to ask an animal what it is feeling.”

1.4.2 Animal preference test methodology

*Free choice method*

The preferences of animals may be observed in an open or closed economy. An open economy refers to a situation where animals are placed in the testing situation for only a limited period of time and therefore can perform behaviour outside the test, whereas a closed economy refers to when animals essentially live in the testing apparatus (Jensen and Pedersen, 2008). A closed economy ensures that animals cannot perform an activity outside the testing environment and this may be particularly important for operant conditioning tasks when the price to access a resource is increased (refer section 1.4.2.3).
The free choice method is the most simple animal preference methodology. This method entails placing an animal into an enclosed area where it has access to two or more environments or resources simultaneously. The animal is then given a period of time with access to both environments or resources, and is not confined in any of the environments at any time. Observations are usually made on the duration of time the animal spends in each environment/resource and sometimes the behaviours performed in each. The preferred environment is commonly deemed as the environment in which the animal spent the majority of its time, however some studies may take into account the behaviours performed in each.

However, depending on the design of this type of preference test, the time spent in a certain area may not provide a true reflection of the animal’s preferences. For example, if compartments contain different resources, the time spent interacting with each may be different due to behavioural requirements with the different resources. For example, an animal may spend a great deal of time in a compartment which provides bedding for resting or sleep, while the animal may spend much less time in a compartment which contains food. This does not necessarily mean that bedding is more important than food.

Y-maze tests

The second type of animal preference test utilises a purpose built apparatus called a Y-maze (or T maze). A Y-maze consists of a start box, which is connected (via a gate) to a ‘choice area’ where the animal can choose to enter one of two resource arms which are connected to the choice area in a Y-shaped fashion (Figure 1.3). (A T-maze is a very similar apparatus; however the resource arms are connected to the choice area in a T-shape). Once the animal has chosen an arm, the animal may then be confined within the arm for a predetermined period. The use of a Y-maze may not only measure the preference of an animal but also the rate at which the Y-maze arm is chosen. This may indicate the level of the animal’s level of motivation to access the resource.
Operant conditioning tasks

An operant conditioning task requires an animal to perform a task in order to gain access to a resource or environment under investigation. Therefore, the animal essentially has to ‘work’ for the resource. Examples of operant tasks include pulling a lever (Holm et al., 2007), pushing a weighted door (Cooper and Appleby, 2003) or pecking a button (Lagadic and Faure, 1987). This adds a quantitative measure to the preference test, as the quantity of work the animal is willing to carry out (as measured by the weight of the door or number of pecks on a button, etc.) may be measured and altered throughout the course of the experiment.

However, the behaviours that animals are required to perform in operant conditioning tasks are relatively unnatural and do depend on the animal’s learning ability to associate the task with resource access. Animals may learn the operant response better for some resources than others (Roper, 1983). For example, animals may learn the association between the task and reward if they are required to perform a relatively ‘natural’ behaviour (McFarland, 1973) or a behaviour that is normally associated with the reward on offer. Young et al. (1994) found pigs that were required
to perform two different operant tasks separately which allowed access to food, responded differently to the tasks. Both tasks required an identical quantity of force to manipulate them. One of the tasks was a panel which pigs had to push while the other was a paddle which the pigs had to chew or root. When the pigs were required to manipulate the paddle in order to gain access to feed, they made more responses and consumed more food in comparison to the panel. This study illustrates that the task itself may influence the animal’s response in an operant conditioning test. In addition, animals may perform the task simply for stimulation rather then a realistic choice. This may especially be the case for animals from a barren environment, although more research is needed (Holm and Ladewig, 2007). These issues with operant conditioning may confound results.

However, an advantage with the operant conditioning preference test is that with the information gathered, it is possible to create a consumer demand curve. Derived from studies in economics, this curve allows experimenters to determine what ‘price’ animals are willing to pay for different resources (Dawkins, 1990). Resources that have inelastic demand are deemed important to the animal, as the animal is willing to keep working to gain access to it, even when the price (e.g. the weight of a door) is increased. For example, the demand for food is inelastic, as it is necessary for the animal’s survival. From an evolutionary perspective, inelastic demand would have evolved in animals to ensure that behaviour that optimised fitness was a priority (Dawkins, 1988). Conversely, elastic demand means that when the price to access the resource is increased, animals are not willing to keep working to gain access (Figure 1.4). Elastic demand would have evolved to ensure animals gain access to ‘luxuries’ (i.e. resources that are indulgent rather than necessary for survival) when the cost to access them is low, but not when the cost to access them is high.
Figure 1.4 A diagram of elastic and inelastic demands. Inelastic demand occurs when animals continue to access a resource even when the amount of work required to access it is increased. Conversely, elastic demand occurs when animals do not access resources as much when the amount of work required to access them increases.

1.4.3 Preference testing of poultry

The preference testing of poultry (predominantly laying hens, broiler chickens and turkeys) have focussed on the housing and environment of the birds. This interest has presumably been due to the welfare issues concerning modern poultry housing systems.

Cage space and flooring

There has been much research conducted on the preferences of laying hens for cage space and flooring type. Many of these studies were conducted in the 1970’s and 1980’s. Numerous studies have found that, when given the choice, hens prefer a larger cage to a smaller cage (Dawkins, 1978; 1981), regardless of genetic strain (Hughes, 1975) or rearing (Dawkins, 1983b; 1983c). Hens also prefer a cage that has a higher roof compared to a lower one when tested singly (Dawkins, 1985b), however, when tested in groups, this preference was not observed (Albentosa and Cooper, 2005). Dawkins (1977a) found that different types of preference tests (free choice versus confinement following a choice (T-maze)) resulted in differing in choice behaviour of hens. In the first experiment, Dawkins (1977a) tested hens in a free choice test between a battery cage and a large pen and found that hens had no preference for either environment. In the second experiment however, hens were tested in T-maze for their choice between a battery cage and a large outside run and results revealed that
hens had a preference for the large outdoor run. Faure (1986; 1994) using groups of hens, found that when they were required to perform an operant conditioning task to increase cage space, there was no clear preference for increased space. Additionally, Faure (1991) found that hens reared in cages were less willing to peck at a button to enlarge their cage in comparison to hens reared in floor pens. As the experiments by Faure (1986; 1991; 1994) were conducted on groups of birds, it is possible that that a single bird performed the operant response to increase size and therefore, the results did not reflect the preference of the entire group. In summary, there is evidence to suggest that hens prefer a larger cage; however the preference of hens for space seems dependent on the type of test utilised.

Laying hen cages have also been scrutinised with regards to the lack of litter. Studies have found a grass floor (Dawkins, 1978; 1983b) or a floor containing litter (Dawkins, 1981) is preferred to wire flooring regardless of experience (Dawkins, 1983b; 1983c). Similarly, Bubier (1996) conducted experiments in which hens were placed in a multi-chambered apparatus, each containing a different resource. The author found that the time hens spent performing foraging behaviours (pecking and scratching) did not changed even if a ‘cost’ (dowels placed at doors of compartments through which birds had to squeeze) was introduced. This suggests that foraging behaviours are important to hens. Conversely, like the results obtained when tested for space in operant conditioning tasks, groups of hens did not show a preference for access to litter when required to perform an operant conditioning task (Lagadic and Faure, 1987; Faure, 1991). Therefore, there are contradictory results in laying hen preferences for flooring. Again, the differences could be due to the type of test, as observed with cage space preference tests.

There have also been a number of studies that have attempted to investigate the importance of both space and flooring type of hens simultaneously in a series of preference tests. Dawkins (1981) determined, by use of a free choice method, that the flooring of a cage was more important than the size of the cage. Hens chose a large cage to a small cage, and a cage with litter to a cage with wire flooring (Figure 1.5). However, when given the choice between a small cage with litter and a large cage with wire flooring, hens preferred the littered cage even if it was as much as four times smaller than the wire cage. In addition, hens chose to enter a cage in which they could barely turn around in order to access litter. Likewise, Dawkins (1978) discovered that hens preferred a small, grass-floored cage to a large, wire-floored cage using a T-maze test.
Test 1 – Large wire cage Vs. Small wire cage.

Test 2 – Wire cage Vs. Cage with litter.

Test 3 – Large wire cage Vs. Small cage with litter.

Figure 1.5 A diagrammatic representation of the study conducted by Dawkins (1981) examining hens’ preference for flooring and space. In a series of tests (1, 2 and 3) the effects of cage type (wire floored versus cage containing a littered floor) and cage size (large cage was four times bigger than the small cage) on the hens’ cage preferences were determined. Arrows indicate hens’ preference.

In conclusion, results from the preference tests of laying hens in regards to cage space, height and flooring are conflicting. It is clear that the type of preference test utilised can affect the results obtained and care must be taken when interpreting such results and comparing results between studies.
Social preferences and group size

The preferred group size of hens relies on a number of factors. Domestic hens have a strong preference to be near familiar hens to unfamiliar hens (Hughes, 1977; Bradshaw, 1992). In comparison, non-domestic species such as the red junglefowl do not have such a strong preference for familiar conspecifics (Vaisanen and Jensen, 2004). In the study by Hughes (1977), it was reported that the group size preferred was highly dependent on the familiarity of the hens in the group. If groups of only unfamiliar hens were available to a test hen, empty cages were chosen most often with the largest group of unfamiliar hens chosen least. However, a study by Dawkins (1982) found an individual hen’s preference for group size and social contact to be variable and dependent on particular conditions. For example hens housed in battery cages or in a large outdoor run with a hen-house preferred a familiar hen to an empty cage and an empty cage to an unfamiliar hen. However, when these hens were used for a subsequent experiment in an attempt to determine the preferred group size of hens, the birds preferred an empty cage to cages with other birds (which varied from one to five familiar birds). To confuse things further, Lindberg and Nicol (1996) obtained results that suggested that the relative dominance ranking of each hen (test and stimulus hens) may influence the preferred group size. The studies by Dawkins (1982) and Lindberg and Nicol (1996) illustrate the need for research into the factors influencing animal choice. In social preference studies, the relationship between test and stimulus animals may be particularly important.

Nest boxes

The pre-laying and nesting motivation of laying hens immediately prior to oviposition has been suggested to be one of the strongest of all motivations (Weeks and Nicol, 2006). In a study by Cooper and Appleby (2003), it was discovered that hens were willing to push a weighted door equally hard to gain access to a nest site 40 minutes prior to oviposition as for food when they were feed deprived for four hours. This study also found that the hens were willing to ‘work’ hardest 20 minutes prior to oviposition. Likewise, Cooper and Appleby (1996) reported that hens were willing to squeeze through narrow gaps in order to gain access to nest sites pre-oviposition. It is the evidence of strong nesting motivation that has been one factor that has driven the addition of nest boxes into some poultry housing systems.
Nest boxes help reduce the number of ‘floor eggs’, which are likely to be broken or dirty in non-cage systems, which may be a significant economic problem (Appleby et al., 2004c). In addition, hens laying in nest boxes may help reduce the incidence of feather and vent pecking and cannibalism in laying hen flocks as individuals are able to escape the pecks from conspecifics post-oviposition when there is a minor prolapse of the oviduct after laying, which acts as an attractive pecking stimulus to other individuals (Savory, 1995).

In order to make a nest box appealing to encourage its use, numerous preference studies have investigated the choices of hens for nest boxes that differed in a number of features. Very few studies have investigated the influence of the position of nest boxes within a cage on the preference by laying hens. The study conducted by Struelens et al. (2005) showed that hens had a distinct preference for a nest box in a particular position. However, the cage position preferred was identical to the position of the nest box which was present in the cage in which they were habituated to the testing situation, making the results questionable. A study on nest box preferences in Japanese quail housed in battery cages found that Japanese quail prefer nest boxes located in the corners of cages compared to those located in the centre (Schmid and Wechsler, 1998). Although information is currently limited, future research into the preferences of nest box position by laying hens may improve the usage of nest boxes.

There have been a number of studies focussing on the substrate presented to laying hens in nest boxes and hens have been found to have definite preferences for substrate. Laying hens prefer to lay on peat or artificial turf to coated wire (Struelens et al., 2005), however, this preference may be influenced by experience. Hughes (1993) reported that naïve hens (which had been raised on wire floors and not yet laid their first egg) preferred to lay on artificial turf to wire floored nest boxes, however, experienced hens showed no preference for nest box flooring, though there was some degree of individual variation. There is also individual variation in the preference for the quantity of litter in a nest box (Petherick et al., 1993a). Astroturf is a more practical nest box flooring for large scale commercial producers due to its lack of dust which may cause health problems due to inhalation (Hughes, 1993), however, Duncan and Kite (1989) reported that hens prefer a nest containing a manipulable substrate in which to nest.

The lighting or the darkness of a nest box is thought to be an important factor on the choice of nest box by laying hens (Appleby et al., 2004c). Appleby et al. (1984) conducted a study looking at the effects of genetic strain (Rhode Island Red strain and a White Leghorn strain) and age of hens (‘pullets’ and ‘mature hens’) on choice for either a high illuminated (40 lux) or low illuminated (5 lux) nest box. Results showed that Rhode Island Red strain
birds preferred an illuminated nest box regardless of age. However the preference of the White Leghorn strain was dependent on age, with the younger birds preferring the low light box and older hens preferring the high illumination box. In contrast, turkey hens have been found to have quite a strong preference for a nest box with a very low light intensity (0.5 lux) (Millam, 1987).

**Lighting**

The lighting of poultry housing is important to the health of the animals as improper or insufficient lighting may lead to physiological and morphological abnormalities such as increased adrenal weight (Siopes et al., 1984), changes in eye morphology and partial or complete blindness in poultry (Harrison et al., 1968; Siopes et al., 1984). For example, Siopes et al., (1984) found that turkeys housed over a two-week period under low lighting (1.1 lux) had reduced body weights, decreased feed intake and changes in eye morphology compared to turkeys housed under higher lighting (either 11, 110 or 220 lux).

Under commercial conditions, caged laying hens are housed under strict light:dark regimes, however the brightness of the lighting may be quite different from farm to farm. In Australia, the Model Code of Practice for the Welfare of Domestic Poultry (Primary Industries Standing Committee, 2002) recommends lighting to be a minimum of 20 lux for chicks in their first three days (to ensure chicks know the location of food and water) and after this period, the lighting may be lowered to as low as 2 lux. In the United Kingdom, the RSPCA’s welfare standards for laying hens states that the lighting in a hen shed may be no less than 10 lux (RSPCA, 2008) while the Department of Environment, Food and Rural Affairs (DEFRA) states that lighting can be at least 5 lux, although preferably 10 lux or above (Department of Environment, Food and Rural Affairs, 2002). Clearly, the lighting standards of hens are inconsistent and preference tests have been aimed at determining the preferred lighting brightness and duration of hens.

Prescott and Wathes (2002) tested laying hens for their choice of illuminance while feeding. They achieved this by conducting two preference tests, a free choice test and an operant conditioning task. Results showed that hens chose to consume food under the brightest light (200 lux) and least preferred to eat under the dimmest light (<1 lux) in the free choice situation. When hens were required to ‘work’ (by pecking at
panels), they were willing to ‘work’ 2.3 times as hard to access food in a lit environment compared to a unlit environment. Similarly, Savory and Duncan (1982) used an operant conditioning technique in a Skinner box to determine whether hens were willing to ‘work’ for light (15 lux) when placed in a dark Skinner box and found that chickens (both a broiler and laying strain) chose to spend approximately 20% of the time in the light. This was in contrast to when the hens were placed in a light Skinner box, as they hardly worked for darkness in this situation. When hens were placed in a Skinner box and allowed to turn the light on or off, they chose to be in light for approximately 80% of their time. These results indicate that hens when feeding prefer bright lighting and also prefer to be in lit environment compared to a dark environment.

In a free choice experiment, Davis et al. (1999) found that young chickens (both broiler and layer strains) had a preference (based on amount of time) for bright lighting (200 lux) when the birds were two weeks of age. However, when the birds reached six weeks of age, their preference had changed to prefer the dimmest lighting on offer (6 lux). The authors also took into account the behaviours performed in each lit compartment. They found that the older birds (six weeks old) preferred to be in the dim environment when performing rather inactive behaviours such as resting and perching. Therefore, differences in the requirements for light and dark are dependant on the motivational state of the bird.

The above results provide evidence that the domestic chicken has preferences for the lighting of its environment. At times the preference does seem to be context-dependent, with factors such as age, behaviour and motivational state influencing their preference.

**Dustbathing substrate**

Poultry have been found to have clear preferences for substrates in which to perform dustbathing behaviour. Laying hens favour peat moss and sand over wood shavings as a dustbathing substrate (van Liere et al., 1990; Santora et al., 1995). Likewise, in a recent study, de Jong et al. (2007) found, using consumer demand theory, that most of the hens were willing to push a weighted door in order to dustbathe when peat moss was presented, while significantly fewer hens were willing to push the door to gain access to sand or wood shavings for dustbathing. In addition to these findings, van Liere et al. (1990) discovered that hens increase dustbathing behaviour.
when they were moved from an environment containing wood shavings to one containing sand, essentially ‘compensating’ for the time with the inferior substrate, further suggesting hens’ preference for sand. Broiler chickens also show this preference, selecting sand for dustbathing behaviour over other substrates on offer (rice hulls, paper and wood shavings) (Shields et al., 2004).

1.5 FACTORS THAT INFLUENCE THE CHOICE BY AN ANIMAL IN A PREFERENCE TEST

1.5.1 Side bias

When an animal is given the choice between two options, a side bias may exist. A side bias may be defined as an animal choosing one option significantly more often than the other (e.g. Y-maze arm) regardless of what options are offered. For example, Petherick et al. (1993) reported that hens given the choice between food and a dustbathing/foraging substrate made more ‘correct’ choices (i.e. selected the resource that they were deprived of) more often on the right Y-maze arm compared to the left. Another side bias has been observed in dairy cows, with certain individual dairy cows within a herd consistently entering one side of a two-door entry milking parlour (Gadbury, 1975; Hopster et al., 1998; Paranhos da Costa and Broom, 2001; Prelle et al., 2004).

Side preferences, along with other lateral preferences such as handedness, are related to the lateralization of the brain. Brain lateralization, which is the where the left and right hemispheres of the brain differ in terms of structure or function (Rogers, 2000), has been found in a range of lower and higher-order vertebrate species (Rogers, 2000).

In vertebrates, lateralization of the brain causes the two hemispheres of the brain to differ in the way they process information and behaviours they control (Rogers, 2009). The right brain hemisphere controls the expression of emotion. In addition, the left eye (which is linked to the right hemisphere) is the eye which is more responsive to potential threats. For example, hens respond to an aerial alarm call by looking up with their left eye (Evans et al., 1993). Moreover, dogs are more responsive to threatening stimuli when presented on the left compared to the right (Siniscalchi et al., 2010). In contrast to the left eye-right brain system, the right eye-left brain system deals with
complex tasks and is the dominant eye used for visual discrimination tasks (e.g. Gunston and Gunston, 1984; Mench and Andrew, 1986). For example, Mench and Andrew (1986) studied domestic chicks that were required to peck for grain on a background of grain-sized pebbles and found that chicks that were permitted to use only their right eye performed the task better (i.e. pecked less at pebbles and more at the grain) in comparison to chicks which could only use their left eye in the task. These differences in eye-brain systems imply animals may predominantly use one eye in a task, which in turn, may lead to a side bias.

1.5.2 Spontaneous alternation behaviour

The first reported observations of spontaneous alternation behaviour (SAB) appeared in psychological literature from the mid 1920’s (e.g. Tolman (1925) and Dennis (1935) cited in Dember and Fowler (1958)). This behavioural phenomenon may be defined as “the tendency for animals to alternate their choice of arms in successive runs of a T- or Y-maze” (Haskell et al., 1998). This means an animal displaying SAB will choose each Y- or T-maze arm alternately, regardless of what the maze arms contain (e.g. whether it contains a reward or nothing). SAB has been widely described in rodents from early psychological studies, most notably mice (e.g. Petchkovsky and Kirkby, 1970) and rats (e.g. Douglas et al., 1973). The motivation for exploratory behaviour, which therefore means the animal sampling its surrounding environment, is likely to be a key causational factor of SAB (Estes and Schoeffer (1955) cited in Haskell et al. (1998)).

Interestingly, there seems to be variation in the occurrence of SAB between species. Several studies have demonstrated the presence of SAB in certain animal species (e.g. guinea pigs (Douglas et al., 1973), domestic cats (Frederickson and Frederickson, 1979) and ferrets (Hughes, 1965)). Several investigations have failed to demonstrate SAB in the young domestic chick (Hayes and Warren, 1963; Dutch, 1969). However, when offered social contact as a reward (in contrast to nothing, food or water), chicks have shown SAB (Cogan et al., 1979). SAB has also not been observed in the mature chicken (Hughes, 1989; Haskell et al., 1998) although there is some degree of individual bird variation.

This high degree of SAB differentiation by species could be influenced by the individual species’ primary sense utilised in exploration. The hens’ predominant sense
in exploration is sight (Syme and Syme, 1975). Therefore, visual contact with the maze arm not chosen in a Y-maze preference trial may be sufficient for chickens to determine that a visit to the arm is not required. Hence, chickens do not have to ‘choose’ a Y-maze arm to explore. In contrast, the rats’ predominant sense in exploration is olfaction (Douglas, 1966). This may mean rats must move into the maze arm (make a choice) to explore it.

1.5.3 Familiarity with the testing environment

The testing environment in animal preference tests, particularly Y- or T-maze tests and operant conditioning tasks, is often novel to the test animal. It has been suggested that domestic hens, in particular, may feel vulnerable in bright open spaces as they have descended from the Jungle fowl whose natural habitat is the dense rainforests of South East Asia (Grigor, 1993 cited in Haskell et al., 1998). Most behavioural studies with the domestic chicken use an evenly lit environment to ensure that there are minimal shadows in the testing environment, which may affect the chicken’s behaviour and elicit a fear response. In addition, animals are usually tested individually. There is evidence to show that the domestic chick shows behavioural and physiological indicators of stress when isolated (Jones, 1984; Jones and Merry, 1988). To ensure that there are no effects on the behaviour of an animal in a novel testing environment (e.g. a Y-maze), animals must be sufficiently familiarised to the novel situation. For example, Krause et al. (2006) ensured the laying hens in their study were properly familiarised to the Y-maze apparatus by placing groups of hens in the Y-maze overnight prior to the day of testing. This ensured that the animals were familiar with the apparatus and eliminated isolation stress by keeping the animals together. In the study by Sherwin (1998) on the lighting preferences of turkeys, the turkeys were tested in groups of three rather than individuals, as the author believed that the turkeys would be “greatly distressed when isolated thus requiring them to be test in a group”. However, interpreting the choice of a group of animals may be difficult as one animal may be selecting the resource/environment and other animals may simply follow their group mates.
1.5.2  Diurnal effects

Animals demonstrate daily cycles of behavioural activity (e.g. feeding, drinking, resting, sleeping) known as circadian rhythms. For example, when permitted, laying hens roost at night time (see section 1.3.2) and research suggests hens work harder to access perches at night in comparison to during the day (Weeks and Nicol, 2006), coinciding with the hens’ roosting time.

Dustbathing behaviour in laying hens is one behaviour shown to occur at certain times during the day. Following a period of dustbathing substrate deprivation, Vestergaard (1982) reported that hens re-allowed dust access in the morning (around 0900 h) had a significantly longer latency to commence dustbathing compared to hen’s re-allowed access in the afternoon (around 1400 h). Therefore, even though the hens had been deprived of dustbathing opportunity, the natural diurnal pattern of dustbathing behaviour may have caused the hens re-allowed access in the morning to be less motivated to commence dustbathing due to the time of day.

Hens’ preference for a nesting site may also be influenced by the time of day. Cooper and Appleby (1995) placed hens in an apparatus in which they could move from a home pen (containing food, water and a perch) to a nest pen (containing an attached nestbox) through an opening in the dividing wall, which could be altered in width. Results showed that hens spent significantly more time in the nest pen in the three hours prior to oviposition, compared to the evening. Therefore, the time relative to oviposition impacted on the hens’ preference for a nesting site.

These examples provide evidence that the time of day that an animal is preference tested may influence their choice. Therefore, depending on what resources are presented in a preference test, the time of day may need to be given consideration when designing preference studies.

1.5.3  Experience

An animal may initially find a novel resource or environment either attractive or aversive which may ultimately affect the animal’s choice behaviour. In most cases, when an animal is presented with a choice between a familiar environment and a novel one, animals tend to prefer the familiar environment.
For example, Petherick et al. (1990) tested the preferences of domestic chicks in a Y-maze for their choice of flooring (wire versus litter). The chicks had been reared on and therefore only had experience with one of the two floorings. Results showed that the chicks preferred the familiar type of flooring (on which they had been reared). Hughes (1976) also reported similar results, with hens preferring the type of flooring to which they were familiar. Likewise, Dawkins (1976) found that the type of housing in which a hen had been raised had an effect on the birds’ rate of choice between a battery cage and an outdoor run, with hens preferring the familiar environment.

The above examples provide evidence that previous experience with the resources presented in a choice test may have significant effects on the preferences of animals and therefore, animals should be provided with experience with all resources/environments prior to be tested.

1.5.4 Motivational state

Motivation may be defined as the internal decision-making process by which an animal chooses to perform a particular behaviour (Barnard, 2004). The motivational state of an animal is based on the internal physiological environment, and the behaviour an animal displays aids the maintenance of homeostasis. The relative importance an animal places on a resource may be dependent on its motivational state. For example, Duncan (1978) used the example of hens that enter trap-nests to lay. Even though the hens are confined within the trap-nest for a period of time (sometimes several hours) after oviposition without access to food, water or social contact, hens repeatedly choose to lay in these devices. Ordinarily, these other resources (i.e. food, water and social contact) would presumably be very important, however the hen’s motivation to search and make a nest prior to oviposition is very high.

A recent review of laying hen priorities and preferences, found that hens’ nesting motivation just prior to oviposition is likely to be the strongest motivation of hens, even out-ranking feed at this time (Weeks and Nicol, 2006). This was found through many preference tests that required hens to ‘work’ to gain access to a nest box at various times before and after oviposition. Examples of these include hens’ willingness to move through extremely narrow gaps (Cooper and Appleby, 1996) and pushing weighted doors (Cooper and Appleby, 2003). This is a clear example how the motivational state of an animal can influence the animals’ preference for a resource.
1.5.5 Stress

Relatively little work has been conducted specifically examining the effect of stress on animal choices. It has been suggested that stress can affect the choice of both humans and non-human animals by increasing their decision speed and perhaps causing the animal to make a decision without taking in all information (Jackson et al., 1980; Keinan, 1987; Mendl, 1999). Mendl (1999) suggests that the learning and memory of animals in preference tests (e.g. learning the association between location and resource presented) may be impaired if the testing environment causes stress to the animals.

Agitated animals may make less clear-cut choices compared to non-agitated animals. For example, pigs confined in a start box of a T-maze for 5 minutes showed agitated behaviour and did not show a preference when allowed to choose between a compartment containing food and an empty compartment. In contrast, pigs held in the start box for 30 seconds did not show agitated behaviour and showed a clear preference for the compartment containing food (van Rooijen and Metz, 1987). However, this study did not specifically examine the effect of agitation on the animal’s stress level.

1.5.6 Genetic effects

Common strains of laying hens in commercial production today are brown (e.g. ISA brown and brown Hyline) and white strains (e.g. White Leghorn and Ross white). White hen strains have been defined as ‘flighty’ (e.g. Murphy, 1977) based on the responses of the birds towards humans. White hens have also been shown to display more fear-related behaviour such as increased tonic immobility (Fraisse and Cockrem, 2006) and increased time to approach a novel food resource (Murphy, 1977). Furthermore, white hens have a higher acute physiological stress response to being handled (Fraisse and Cockrem, 2006). In addition, Petherick et al. (1990b) reported that brown hens were more successful at learning the position of peat moss in a Y-maze compared to white hens. Therefore, these strains may also behave differently in preference studies.

Few studies have examined the effect of hen strain on preferences. Hughes (1976) found no strain differences in hens’ preference for cage flooring (wire or litter). Conversely, Appleby et al. (1984) found that the strain of hen influenced their choice
for nest box illumination, however, age also impacted on their preference (refer section 1.4.3.3)

With the little research conducted examining the effects of strain, this is clearly an area for further research to determine the effects of genetic strain on preference.

1.5.7 Incentives of resources

In a Y-maze test (and some other preference methodologies) animals usually, but not necessarily, have a pre-determined period of time with which they have contact with their chosen resource. This period of time may be defined as the quantity of resource. If the quantity of resource is too long, the animal may experience satiety. Alternatively, if the amount of time is too short, the animal may become frustrated due to the inability to perform or interruption of behavioural requirements with the resource (e.g. dustbathing or sleep). Therefore, an adequate amount of time must be provided. The period of confinement may be determined by observing the interaction of the animal with the resources under investigation. For example, a complete dustbathing bout takes, on average, 27 minutes (Vestergaard, 1982), so if a dustbathing substrate is provided, this should be taken into consideration.

Few studies have investigated the effects of altering reward quantities in preference studies. Jensen et al. (2004) conducted an operant conditioning task in which dairy cows had to press a panel for access to rest while in tether stalls. The experiment investigated the effects of reward duration of rest on dairy cows. Results revealed that the longer reward (20 minutes rest) was more valuable to the cows compared to the shorter reward (10 minutes rest) following a long period (6 hours) of rest deprivation. Jensen et al. (2004) also suggested that the shorter reward may not have been as valuable due to the interruption of resting (after the 10 minutes of reward) compared to the longer reward.

Like the quantity of resource, the quality of resource may also affect how attractive it is perceived. The term quality refers to ‘characteristics with respect to excellence’ (Heinemann Australian Dictionary, 1995). For example, with respect to dustbathing substrates, quality may relate to the effectiveness of the material for its function. Research has shown that substrate which are composed of a smaller particle size are more effective in terms of reaching the proximal (plumulous) part of the feathers and thus provide a more effective dustbathing bout (van Liere and Siard, 1991).
1.5.8 Handling

Handling animals is an essential part of most preference tests, as the animals must be moved to and from experimental apparatus. Most preference tests require animals to be placed in a novel environment, in which the animal can make a choice. It has been proposed that the chicken’s normal reaction to humans is fear (Duncan, 1990). If an animal has a high level of fear, this can impact on behaviour. For example, fear in the laying hen can lead to increased tonic immobility and withdrawal from humans, however, this level of fear may be reduced by regular gentle handling (Jones and Faure, 1981; Jones, 1994) but not regular rough handling (Jones, 1992). Barnett et al. (1994) reported that hens that received regular human contact had a reduced avoidance of humans and a lower plasma corticosterone response to being held by a human compared to hens that received minimal handling. Even if only one individual person has performed the handling procedure with the animals, the animal’s fear of humans is reduced and is generalised to other humans as well (Jones, 1994).

Therefore, animals that are to be handled by humans in experiments should be thoroughly familiarised to being handled to reduce their fear response to humans.

1.5.9 Social factors

The majority of domestic animals are gregarious and therefore live in close contact with conspecifics in large herds or flocks. Moreover, there is evidence to suggest that humans and non-human animals may make decisions as a group rather than as individuals (e.g. Conradt and Roper, 2005; Conradt and List, 2009; King and Cowlishaw, 2009). However, animals are commonly tested in isolation in behavioural studies, including preference tests. This raises the question; does this social isolation during preference testing affect the animals’ motivation and choice for resources on offer? Sherwin and Nicol (1998) were one of the first to suggest that the social context may unwittingly influence the behaviour of an animal in an operant conditioning task. Since then, it has been discovered that pigs are willing to work harder for a food reward in the presence of another pig, essentially making food ‘more valuable’ when a conspecific is present (Pedersen et al., 2002). In contrast, the motivation of laboratory mice to gain access to a running wheel has been found to greatly reduce when in the presence of cage mates in comparison to when the mouse was in isolation (Sherwin,
The above two examples illustrate that the social context in a preference test may affect the motivation of the test animal to gain access to a particular resource. It is generally considered that animals do react differently when tested singly or as a group (Olsson, 2007).

1.6 EXPERIMENTAL PLAN

The aim of the thesis was to examine the effects of several factors of Y-maze preference test design on the motivation and consequent choice behaviour of laying hens. The factors chosen to be studied were aspects of design which have previously been proposed to have an affect on animal motivation, but as yet have not been thoroughly examined.

The first aspect of preference test design was the quantity of resource (i.e. time with the resource), a factor that has been proposed to impact on how an animal perceives a resource presented in a preference test (Nicol, 1997; Kirkden and Pajor, 2006; Fraser, 2008). While the quantity of resource has been studied using demand tasks (Jensen et al., 2004), it has not been specifically examined in Y-maze test methodology.

The resources presented in the Y-maze were a dustbathing/foraging substrate and social contact. These resources were selected over food, as food may be the ‘gold standard’ (e.g. food has been found to be relatively inelastic in operant conditioning studies, e.g. Faure and Lagadic (1994)), and thus its use may mask subtle effects on choice behaviour.

The next two experiments carried on from the first experiment, examining the effects of interrupting dustbathing bouts and examining the learning capability of hens in the Y-maze.

The final experiment was similar to the first, however, it examined two other factors in the design of Y-maze methodology; interval of testing and quality of resource (i.e. type of dustbathing substrate). Again, social contact and a dustbathing/foraging substrate were the resources presented in the Y-maze.
CHAPTER 2

THE EFFECTS OF QUANTITY OF RESOURCE ON
THE CHOICE BEHAVIOUR OF LAYING HENS FOR
PEAT MOSS AND SOCIAL CONTACT

2.1 INTRODUCTION

In a Y-maze preference test, an animal is able to make a choice between two resources. Once the animal has made a choice, it is commonly confined with the selected resource for a pre-determined period of time. This amount of time can be defined as the quantity of reward. While it has previously been suggested that, for experimental animals, the quantity of reward in a preference test may affect how an animal perceives a resource (Nicol, 1997; Kirkden and Pajor, 2006; Fraser, 2008) and thus potentially its preference, this concept has yet to be examined in Y-maze preference tests. For example, Kirkden and Pajor (2006) suggested that restricting the reward size in choice tests may devalue the resource. Results from operant conditioning tasks have demonstrated that altering reward duration can affect the demand function (Jensen et al., 2004). Therefore, investigating this factor in Y-maze tests may prove to be valuable for the selection of resource quantities in future research and thus make Y-maze tests a more rigorous technique at assessing the preferences of animals.

To date, the scientific literature lacks consensus on resource quantities and this factor has been varied between studies (e.g. 10 minutes (Lingberg and Nicol, 1996); 2 minutes (Haskell et al., 1998); or 1 minute (except for last trial of 30 minutes to allow for dustbathing) (Petherick et al., 1993b)). Early environmental preference studies by Dawkins (1977a, 1981, 1982) used a resource quantity of 5 minutes. The reasoning behind this was stated simply as to “ensure that the hen had a standard and measured amount of time in the environment of her choice” (Dawkins, 1977a). However, many studies fail to describe how or why the quantity of resource was chosen (e.g. Hughes, 1976; Dawkins, 1978; Lingberg and Nicol, 1996). This suggests that the quantity of resource in some studies may have been chosen for experimental convenience rather
than considering behavioural requirements of the animal with presented resources (e.g. dustbathing, feeding, etc.).

This experiment aimed to determine the effects of quantity of a peat moss reward on the motivation and choice behaviour of laying hens in a Y-maze preference test where they were offered the choice between a peat moss (a dust substrate) and social contact.

Dustbathing is a series of behavioural components that aids the maintenance of plumage (van Liere and Bokma, 1987; Olsson and Keeling, 2005). On average, the duration of a dustbathing bout is 27 minutes and occurs every second day with peak activity being around midday (Vestergaard, 1982). Laying hens have been found to have preferred substrates in which to dustbathe, with peat moss being the most preferred material (Petherick and Duncan, 1989; de Jong et al., 2007).

Laying hens are highly social animals that flock together and form a stable dominance hierarchy, which may remain stable for a number of years (Schjelderup-Ebbe, 1922 cited in Mench and Keeling, 2001). Social contact is presumably important for laying hens as many behaviours, such as feeding, dustbathing and preening are often performed in synchrony by many individuals (Hughes, 1971; Webster and Hurnik, 1994; Duncan et al., 1998). Additionally, social isolation is known to cause stress, both behaviourally and physiologically, in domestic chicks (Jones and Merry, 1988).

Therefore, both social contact and peat moss appear to be important resources for laying hens. The hypothesis for the experiment was that altering the quantity of reward would influence how attractive a resource was perceived to be by laying hens and this would ultimately affect motivation and choice behaviour. To test this hypothesis, hens were given a choice in a Y-maze for access to peat moss for three different periods of time and a familiar, subordinate hen for a fixed period of time.
2.2 MATERIALS AND METHODS

2.2.1 Animals and housing

Selection of experimental animals

Thirty hens (*Gallus gallus domesticus*, Hy-line Brown laying strain) were chosen from a flock of 78 beak-trimmed hens group-housed in cages (1.14 m x 0.50 m x 0.45 m) with six to eight hens per cage. These cages were within the experimental facility and the birds had been residing in them since 20 weeks of age. When the hens were 27-weeks-old, a plastic tray (0.46 m x 0.24m x 0.03 m) was placed in each group cage and was filled daily with peat moss (Canadian TE-EM sphagnum peat moss).

Birds were selected for the experiment at 32 weeks of age based on their social status, defined as either ‘dominant’ or ‘subordinate’. This technique was selected to determine the social status of some of the hens, as it has been successful in the past, so long as the feed deprivation period is relatively long (e.g. Banks *et al.*, 1979). Individual hens were identified via coloured leg bands and were assessed during 5-minute direct observations of each cage when feed (a commercial layer pellet) access was provided following overnight feed deprivation (mean of 19.10 h deprivation, range 18.85 to 19.25 h). Feed deprivation was carried out by covering each cage’s feed trough with two metal covers. When each cage of hens was provided with feed access, only one metal cover was removed thus reducing the space of the feed trough (to approximately 0.57 m of feed trough length), which did not provide enough space for all birds in the cage to feed simultaneously. Observations were made each morning, commencing at 1100 h, over five consecutive days. The order of cage observations was randomised each day. Each time a hen was observed displaying a behaviour of interest, the event was recorded. Behaviours defined as dominant were: a hen pecked another individual at the feed trough or the hen easily regained access to feed. Behaviours defined as subordinate were: hen was the recipient of a peck at the feed trough, did not easily regain access to feed and/or paced or vocalised while attempting to gain access. At the end of the observation period, individuals from the same cage that were consistently recorded as dominant and subordinate were chosen as a pair. Fifteen pairs of hens were selected for the experiment.
Selected hens were housed individually in cages (0.57 m x 0.50 m x 0.48 m). Each cage contained an external feed trough at the front of the cage and one to two nipple drinkers at the rear. Hens had *ad libitum* feed and water in their home cage throughout the experiment. Each cage also contained a plastic tray identical to those that were in the original group cages. The tray was filled daily with approximately 400 mL peat moss. Peat moss was chosen as the substrate, as it has been found to be a preferred substrate for dustbathing by laying hens (Petherick and Duncan, 1989; de Jong *et al.*, 2007). Each hen pair (i.e. a dominant and a subordinate hen chosen from the same group housing cage) was randomly allocated into a pair of cages. Pairs of cages were separated by metal mesh, which allowed limited tactile contact between neighbours. Each pair of cages was separated from other pairs by solid walls. Dominant hens were chosen to be test subjects for the experiment while the subordinate neighbour of each dominant hen was designated to be the ‘social option’ in the Y-maze for the dominant individual during testing. This pairing was determined to ensure the ‘social option’ in Y-maze testing was attractive and non-threatening to the test bird, as it has previously been demonstrated that hens will avoid unfamiliar hens or familiar hens that are dominant to them (Dawkins, 1982; Bradshaw, 1992; Grigor *et al.*, 1995). From here onwards dominant hens will be referred to as test hens and subordinate hens will be referred to as non-test hens.

Hens were checked and fed, and the tray refilled with peat moss at approximately 1030 h each morning. The hens were kept in an environmentally-controlled shed at a temperature of about 21°C (mean 20.9°C, range 18.4°C to 28.4°C) and on a 12:12 light:dark cycle with lights coming on at 0700 h.

### 2.2.2 Video observations

Video recordings were made of hens in their home cages for 12 days prior to the commencement of pre-experimental handling. One video camera (black and white digital CCD) was fixed approximately 0.6 m in front of each of the test hens’ home cages on metal poles. The real-time recordings were made continuously for the 12-day period. Only the recordings of ‘dustbathers’ and ‘non-dustbathers’ were reviewed (see Section 2.2.6). For the one-hour period following the daily refilling of the tray with peat moss, the occurrences of dustbathing in the peat moss and sham dustbathing (i.e. dustbathing on
wire, not incorporating peat moss as the dustbathing substrate) and the latency to dustbathe were recorded. Latency to dustbathe was defined as the time from when the tray was filled with peat moss to the time when the hen commenced dustbathing behaviour. Only 11 of the 12 days of the pre-experimental period were used because on one day (the fifth day), the dustbaths were refilled before 1000 h and several hens laid in the subsequent hour, which affected their dustbathing behaviour.

2.2.3 Pre-experimental handling

To ensure hens were accustomed to being handled, all hens (including non-test birds) were given one minute of handling twice a day for seven days. Hens were handled at approximately 1100 h and 1600 h each day. These times were selected so as to increase the likelihood that hens were post-oviposition. If a hen was showing signs of pre-laying behaviour she was not handled until she had laid. Initially the cage door was opened and the hen was touched. Over the week, the intensity of the handling increased such that, by the end of the week, each bird was picked up and held outside their home cage before being returned. Pre-experimental handling was conducted in the week immediately prior to the commencement of the runway test.

2.2.4 Runway test

As the Y-maze test in this experiment included social contact as a resource, the first part of this experiment aimed to assess the hens’ preference for a familiar or unfamiliar hen in a runway test.

Runway apparatus

The runway apparatus is shown in Figure 2.1. The walls of the runway were 0.50 m high and the roof consisted of five removable metal mesh roof pieces (one over each end compartment and each zone). It comprised a main runway with a compartment at each end (B) in which stimulus birds were placed. The compartment was separated from the main runway by a metal mesh divider. The runway was separated into three virtual zones; the middle zone (‘M’ zone), familiar zone (i.e. the zone closest to the familiar hen compartment, ‘F’ zone) and the unfamiliar zone (i.e. the zone closest to the unfamiliar
hen compartment, ‘U’ zone). Two cameras (black and white digital CCD) were mounted approximately 1.3 m above the runway to provide a view of the three zones from overhead.

![Diagram of runway apparatus]

**Figure 2.1** Runway apparatus

*Runway test procedure*

Each test and non-test hen was familiarised to the runway once a day on three consecutive days. Familiarisation was conducted by placing each individual bird in the centre of the runway (for test hens) or one of the end compartments (for non-test hens) and leaving her in the runway for 30 seconds. Each test and non-test hen was familiarised to the runway apparatus individually. The order of familiarisation was randomised each day.

All testing was conducted after 1100 h to increase the likelihood that hens were post-oviposition to avoid pre-laying behaviour. If a hen was showing signs of pre-laying behaviour, she was left for testing until the end of the scheduled testing for that day.
For each runway test a non-test hen was placed in each of the end compartments 30 seconds prior to the test hen being placed in the middle zone. One of these non-test birds was the test hen’s neighbour (i.e. a familiar, subordinate bird), while the other was a pseudo-randomly chosen non-test bird, which was chosen so as to ensure that the hen was unfamiliar to the test hen. Unfamiliar stimulus (non-test) hens in the runway test were chosen so as to minimise the chance that the test birds had had visual contact with the stimulus hen. Even though the test hen may have had auditory and olfactory contact with the unfamiliar hen, research has showed that visual contact is necessary for individual recognition in the chicken (e.g. Dawkins, 1995; D’Eath and Stone, 1999; Hauser and Huber-Eicher, 2004). Unfamiliar, non-test hens were chosen at random so long as the hen was from a different tier of cages from that of the test hen, was housed at least one pair of cages across (horizontally) from that of the test hen, had not been used in the runway trial immediately prior to or subsequent to the runway test (i.e. non-test birds were not used in two consecutive runway trials) and had not previously been utilised as an unfamiliar non-test hen for the test hen. The ends at which the familiar and unfamiliar non-test hens were placed were randomly determined for each hen in each runway test.

In a randomly determined order, each test hen was placed in the runway in the ‘M’ zone and left in the runway for three minutes. The side of the runway apparatus in which the hen was placed in the runway was randomly determined. Runway tests were conducted three times a week (Monday, Wednesday and Friday) over two weeks for a total of six runway tests per bird.

Data collated from real-time digital video recordings were the times spent in each of the three zones (seconds). Due to technical problems the first day of runway tests was not recorded, therefore five days of data were collected.

Using results from the runway test, a familiarity score was calculated for each test hen. The familiarity score was calculated by subtracting the proportion of time the hen spent in the ‘M’ zone from the proportion of time the hen spent in the ‘F’ zone, using data from all five tests. Familiarity scores ranged from –1 to 1, with a score closer to 1 meaning that the hen spent more time near the familiar than the unfamiliar hen.
2.2.5 Preference testing

Y-maze apparatus

The Y-maze was a purpose built apparatus constructed from galvanised steel. The floor and removable roof pieces were constructed out of mesh, while the walls (0.49 m high) were constructed out of a solid metal sheet (Figure 2.2). The start box had a mesh gate and walls, thereby providing birds with a view of the contents of each arm prior to being released from the start box. The gates between the choice area and each arm were solid metal. The mesh flooring of the maze was identical to the flooring of the hens’ home cage, while the start box walls and roof piece were a smaller mesh.

All the gates in the Y-maze were fixed to cords and run through pulleys so that all gates could be opened and closed by one operator standing behind the start box.

![Y-maze apparatus diagram]

Figure 2.2 Y-maze apparatus

Y-maze familiarisation

Test hens were familiarised with the Y-maze once per day on three consecutive days, commencing at 1100 h. In a randomly determined order, each hen was placed in the
Start box (SB) of the Y-maze with both arm gates open and no resources present. After 10 seconds the SB gate was opened and the hen was allowed to leave. If the hen had not left within 30 seconds, she was gently pushed forward (with a flat hand on the tail). The SB gate was closed once she had left the SB. Each hen was given a total of three minutes in the Y-maze, once she had left the SB, to freely explore the apparatus.

Y-maze training

Throughout Y-maze training and testing, hens were socially restricted in their home cage by placing opaque rubber partitions between pairs of cages and trays of peat moss were removed from hens’ home cages.

Test hens were randomly allocated to one of three treatments (n=5), which differed in the amount of time hens had access to peat moss in the Y-maze. The treatments were ‘Short’ (2 minutes access), ‘Intermediate’ (20 minutes access) and ‘Long’ (45 minutes access). These durations of reward were based around the average duration of a dustbathing bout in laying hens of 27 minutes (Vestergaard, 1982). The Long treatment was considered sufficient time to complete a dustbathing bout, the Short treatment was considered as a more practical reward quantity (e.g. this reward quantity would allow a large number of experimental animals to be tested over a shorter period) and the Intermediate treatment was chosen as a reward quantity intermediate to the other treatments. The quantity of time for social contact in the Y-maze remained constant at 5 minutes for all hens. It was decided to keep the social contact reward constant so as to simplify the experiment.

Each hen was randomly allocated one Y-maze arm (left or right) in which the tray of peat moss was always placed. The tray was identical to that which the birds had in their home cage and was refilled with peat moss after every training/testing trial. The other arm contained the test hen’s neighbour, which was confined in a mesh-fronted box at the end of the Y-maze arm. If the Y-maze arm in which the social option was placed had previously contained the tray of peat moss, the arm was cleaned, by brushing, as to ensure no peat moss remained in the arm.

All birds were trained individually, in a randomly determined order on alternate days for five training sessions per bird. The training sessions aimed to ensure that each hen had equal contact with each Y-maze arm so that the hen learnt what each contained and the quantity of reward for each. To accomplish this, in each training session each hen
received two consecutive trials in the Y-maze, in which only one Y-maze arm was open at a time. The arm in which the bird was first allowed access was determined randomly for each bird each training day.

Each hen was placed in the SB for 10 seconds before the SB gate was opened. The hen was allowed 30 seconds to leave the SB and if she did not leave voluntarily, she was gently pushed forward into the choice area. The SB gate was closed after the hen had moved out. The hen was then given another 30 seconds to move into the open arm and if she had not, she was gently pushed towards the arm. This continued until the hen had entered the arm. Once the hen entered the arm, the arm gate remained open (i.e. providing access to both the arm and choice area). The hen remained in the Y-maze for the designated time (i.e. 5 minutes for social contact or the period of the treatment for peat moss) before either being returned to the SB for the second consecutive training trial or being returned to her home cage.

**Y-maze testing**

The Y-maze testing procedure was similar to that for training, but both Y-maze arm gates were open when the hen was in the SB. When the hen moved out of the SB and entered one of the Y-maze arms, the gate of the arm not chosen was closed and the hen remained in the maze for the required period (i.e. the treatment duration if peat moss was chosen or five minutes if the hen chose the social contact arm).

Each test hen had one Y-maze test trial per day on alternate days for a total of eight test trials. The times to choice, Y-maze arm and resource chosen were recorded by direct observation. The time to choice was defined as the duration from when the SB gate was opened until the hen had entered one of the arms (i.e. moved past the position of the arm gate (Figure 2.2)). Additionally, whether a dustbathing bout commenced as well as the duration of dustbathing (seconds) were recorded by direct observation. A dustbathing bout was defined to commence when the hen first rolled onto her side and was recorded as completed when the hen stood up and shook her feathers. Whether or not the dustbathing bout was interrupted (i.e. the bird was picked up and taken back to her home cage prior to completion of the dustbathing bout) was also recorded.
2.2.6 Assessment of individual variation

To explore individual variation in dustbathing behaviour, birds were classified as one of three types, based on the occurrence of dustbathing activity in the Y-maze. When hens chose peat moss, every bird firstly performed foraging behaviour (i.e. pecking and scratching), after this only some hens dustbathed (as defined above). Therefore, the three types of birds were a ‘dustbather’ (a hen which, when peat moss was chosen, commenced a dustbathing bout on >85% of Y-maze trials (i.e. the hen performed mostly dustbathing behaviour when peat moss was chosen)); a ‘non-dustbather’ (a hen which, when peat moss was chosen, commenced a dustbathing bout on <15% of Y-maze trials (i.e. the hen performed mostly foraging behaviour when peat moss was chosen)); or an ‘other’ (a hen which, when peat moss was chosen, commenced a dustbathing bout on 15-85% of Y-maze trials).

Once hens were classified as one of the types of birds as described above, the pre-experimental video recordings were reviewed and aspects of the birds’ dustbathing behaviour during the hour following the home cage trays being filled with peat moss were recorded (see section 2.2.2).

2.2.7 Statistical analysis

For each bird, the effect of treatment (quantity of resource) on the proportion of trials that peat moss was chosen was analysed as a generalised linear model with over-dispersed errors and logistic link function. Time to choice (all trials) and time to choice when peat moss was chosen were averaged over the eight trials for each hen. After negative reciprocal transformation, these two averaged variables were analysed as one-way analyses of variance, with an indicator covariate for whether the peat moss was on the left or right side of the Y-maze. Individual birds were the experimental unit of analysis in all statistical analyses.

A parsimonious general linear model relating the logarithm of average duration of dustbathing bouts (performed in the Y-maze during testing) plus 100 to familiarity score and treatment was developed. Parsimonious logistic over-dispersed generalised linear models relating the proportion of peat-chosen trials a dustbathing bout occurred and the proportion of dustbathing bouts (in the Y-maze) that were interrupted to familiarity score and treatment were developed.
The video data (pre-experimental home cage observations) collected for hens classified as ‘dustbathers’ and ‘non-dustbather’ was compared using by Student’s t-test.

2.3 RESULTS

2.3.1 Runway test

The individual hen familiarity scores obtained from the runway test are presented in Table 2.1. The familiarity scores ranged from approximately -0.25 to 0.38.

<table>
<thead>
<tr>
<th>Bird number</th>
<th>Treatment</th>
<th>Familiarity score</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>Intermediate</td>
<td>-0.255856</td>
</tr>
<tr>
<td>6</td>
<td>Short</td>
<td>-0.185056</td>
</tr>
<tr>
<td>4</td>
<td>Intermediate</td>
<td>-0.180400</td>
</tr>
<tr>
<td>11</td>
<td>Short</td>
<td>-0.146811</td>
</tr>
<tr>
<td>2</td>
<td>Short</td>
<td>-0.118322</td>
</tr>
<tr>
<td>15</td>
<td>Intermediate</td>
<td>-0.01523</td>
</tr>
<tr>
<td>14</td>
<td>Long</td>
<td>-0.014511</td>
</tr>
<tr>
<td>10</td>
<td>Short</td>
<td>-0.008100</td>
</tr>
<tr>
<td>8</td>
<td>Long</td>
<td>0.036644</td>
</tr>
<tr>
<td>12</td>
<td>Intermediate</td>
<td>0.044311</td>
</tr>
<tr>
<td>5</td>
<td>Intermediate</td>
<td>0.063422</td>
</tr>
<tr>
<td>13</td>
<td>Long</td>
<td>0.070456</td>
</tr>
<tr>
<td>1</td>
<td>Long</td>
<td>0.097233</td>
</tr>
<tr>
<td>9</td>
<td>Short</td>
<td>0.154078</td>
</tr>
<tr>
<td>7</td>
<td>Long</td>
<td>0.379778</td>
</tr>
</tbody>
</table>
2.3.2 Choice behaviour, time to choice and dustbathing activity in the Y-maze

Fourteen of the fifteen hens chose peat moss on at least seven of their eight trials. One hen in the Intermediate treatment chose social contact and peat moss an equal number of times. There was a tendency ($P = 0.07$) for birds in the Intermediate treatment to choose peat moss less often compared to both the Long and Short treatments (Table 2.2). Hens in the Intermediate treatment were slower to move through the Y-maze in all trials ($P = 0.0015$) and trials where peat moss was chosen ($P = 0.0009$) compared to the other treatments (Table 2.2).

There was also a significant effect of treatment on the occurrence and duration of dustbathing bouts, as well as the proportion of bouts that were interrupted (Table 2.2). The Long treatment hens commenced a dustbathing bout on more peat-chosen trials ($P = 0.035$) and had a longer bout duration ($P = 0.00025$) compared to both the Intermediate and Short treatment hens. Long treatment hens also had a significantly lower proportion of their dustbathing bouts interrupted ($P = 0.0079$) when compared to the other treatments (Table 2.2).

The raw data for the duration for interrupted and complete bouts for each treatment is presented in Table 2.3.
Table 2.2  Summary of choice behaviour, time to choice and Y-maze dustbathing behaviour data. Bold P-values indicate significant differences.

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Covariate</th>
<th>Transformation</th>
<th>Transformed mean/proportion (Back transformed mean/proportion)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proportion of trials dust was chosen</td>
<td>-</td>
<td>Log odds a</td>
<td>3.66 (0.98) 1.55 (0.83) 3.66 (0.98) 1.206-1.577 0.07</td>
<td></td>
</tr>
<tr>
<td>Time to choice (all trials) (seconds)</td>
<td>Side of dustbath</td>
<td>Negative reciprocal</td>
<td>-0.47 (2.13) -0.24 (4.17) -0.54 (1.85) 0.066 0.0015</td>
<td></td>
</tr>
<tr>
<td>Time to choice when dust chosen (seconds)</td>
<td>Side of dustbath</td>
<td>Negative reciprocal</td>
<td>-0.46 (2.17) -0.25 (4.00) -0.56 (1.78) 0.062 0.0009</td>
<td></td>
</tr>
<tr>
<td>Proportion of dust-chosen trials a dustbathing bout was commenced</td>
<td>Familiarity score</td>
<td>Log odds a</td>
<td>-2.05 (0.11) -0.78 (0.31) 1.68 (0.84) 1.13-1.43 0.035</td>
<td></td>
</tr>
<tr>
<td>Proportion of dust-chosen trials in which a dustbathing bout was interrupted</td>
<td>Familiarity score</td>
<td>Log odds a</td>
<td>∞ (1.00) 9.88 (0.995) -7.53 (0.17) 7.07 (Intermediate vs. Long only) 0.0079</td>
<td></td>
</tr>
<tr>
<td>Duration of dustbathing bouts b (seconds)</td>
<td>Familiarity score</td>
<td>Log_{10}(y+100)</td>
<td>1.95 (-12) 2.38 (142) 3.00 (897) 0.164 0.00025</td>
<td></td>
</tr>
</tbody>
</table>

a  log odds = loge(p/(1-p)) where p is the proportion being estimated  
b  Duration of all dustbathing bouts when dust was chosen, including a value of 0 when a dustbathing bout was not commenced
<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dustbathing bout type</th>
<th>Number of bouts / total number of bouts</th>
<th>Mean duration of dustbathing bout (seconds)</th>
<th>Standard deviation (seconds)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Short</td>
<td>Interrupted</td>
<td>10 / 10</td>
<td>61.3</td>
<td>33.03</td>
</tr>
<tr>
<td>Intermediate</td>
<td>Interrupted</td>
<td>11 / 17</td>
<td>821.27</td>
<td>244.78</td>
</tr>
<tr>
<td></td>
<td>Complete</td>
<td>6 / 17</td>
<td>618.67</td>
<td>263.52</td>
</tr>
<tr>
<td>Long</td>
<td>Interrupted</td>
<td>7 / 24</td>
<td>691.7</td>
<td>591.71</td>
</tr>
<tr>
<td></td>
<td>Complete</td>
<td>17 / 24</td>
<td>1211.65</td>
<td>298.28</td>
</tr>
</tbody>
</table>

2.3.3 The relationship between familiarity score and dustbathing activity

Within a treatment, the proportion of peat-chosen trials in which a dustbathing bout occurred (Figure 2.3) and the mean duration of dustbathing in Intermediate and Long treatment hens (Figure 2.4) strongly decreased with increased familiarity score, whilst the proportion of dustbathing bouts that were interrupted strongly increased with familiarity score (Figure 2.5). For birds with similar familiarity scores, the proportion of peat-chosen trials in which a dustbathing bout occurred (Figure 2.3) and the duration of dustbathing bouts (Figure 2.4) increased with an increased duration of peat moss access and the proportion of dustbathing bouts that were interrupted (Figure 2.5) decreased with an increased time of peat moss access.
Figure 2.3  Relationship between the proportion of dust-chosen trials a dustbathing bout occurred and the familiarity score for Short (♦), Intermediate (▲) and Long (●) treatments.
Figure 2.4  Relationship between the mean duration of dustbathing bouts and the familiarity score for Short (♦), Intermediate (▲) and Long (■) treatments. A separate P value is presented for each treatment because the model for average duration includes a term for a treatment by familiarity score interaction.
Figure 2.5  Relationship between the proportion of dustbathing bouts that were interrupted and the familiarity score for Short (♦), Intermediate (▲) and Long (■) treatments. Note: due to some hens not engaging in dustbathing behaviour, some treatments do not have five points.

P Values:
Treatment: $P = 0.0079$
Farm Bird Score: $P = 0.011$
2.3.3 Individual variation

Of the 15 hens, four were identified as dustbathers, while six were identified as non-dustbathers (Figure 2.6). The treatments to which these birds were allocated to is shown in Table 2.4. During the hour following the refilling of dustbaths in the pre-experimental period, birds defined as dustbathers performed dustbathing bouts on more days ($P = 0.0135$) and had a shorter latency to commence dustbathing ($P = 0.0043$) when compared to birds defined as non-dustbathers (Table 2.5). In addition, dustbathers had a significantly higher proportion of dustbathing in the peat moss substrate (as opposed to sham dustbathing bouts) compared to non-dustbathers ($P = 0.0032$). There was no difference in the mean duration of dustbathing bouts between dustbathers and non-dustbathers ($P = 0.4585$).

![Figure 2.6](image)

**Figure 2.6** Histogram of the proportion of Y-maze test trials in which a dustbathing bout was commenced. DB = hens defined as dustbathers (hens that dustbathed on >85% of peat-chosen Y-maze trials), NDB = hens defined as non-dustbathers (hens that dustbathed on <15% of peat-chosen Y-maze trials).
Table 2.4  Distribution of hens defined as dustbathers and non-dustbathers between treatments.

<table>
<thead>
<tr>
<th>Dustbathers</th>
<th>Non-dustbathers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bird number</td>
<td>Treatment</td>
</tr>
<tr>
<td>3</td>
<td>Intermediate</td>
</tr>
<tr>
<td>6</td>
<td>Short</td>
</tr>
<tr>
<td>8</td>
<td>Long</td>
</tr>
<tr>
<td>13</td>
<td>Long</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 2.5  Dustbathing activity in the hour following the refilling of the hens’ home cage dustbath during 11-day pre-experimental video observation period. Type of hen was determined from dustbathing activity in the Y-maze, (hens defined as ‘dustbathers’ commenced a dustbathing bout on >85% of peat-chosen Y-maze trials and hens defined as ‘non-dustbathers’ commenced a dustbathing bout on <15% of peat-chosen Y-maze trials). Bold P-values indicate significant differences, df=8.

<table>
<thead>
<tr>
<th>Type of hen</th>
<th>SED</th>
<th>t-value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dustbather</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-dustbather</td>
<td>426.43</td>
<td>3.9322</td>
<td>0.0043</td>
</tr>
<tr>
<td>n</td>
<td>4</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Latency to dustbathe (seconds) (when dustbathing occurred)</td>
<td>737.92</td>
<td>2414.75</td>
<td>426.43</td>
</tr>
<tr>
<td>Proportion of days dustbathing occurred within an hour of refilling the dustbath</td>
<td>0.7955</td>
<td>0.3737</td>
<td>0.134</td>
</tr>
<tr>
<td>Proportion of dustbathing bouts in peat moss (as opposed to sham dustbathing)</td>
<td>0.773</td>
<td>0.146</td>
<td>0.151</td>
</tr>
<tr>
<td>Mean duration of dustbathing bouts (including real and sham bouts) (seconds)</td>
<td>1632</td>
<td>2057</td>
<td>545.64</td>
</tr>
</tbody>
</table>
2.4 DISCUSSION

The results from this experiment revealed that all hens preferred peat moss to social contact, regardless of the quantity of resource provided. However, hens receiving 20 minutes of peat moss access in the Y-maze had a tendency to select peat moss less often and were significantly slower to move through the Y-maze compared to hens receiving 2 or 45 minutes of peat moss access.

The runway test indicated that there was variation between hens in the proportion of time they spent near the familiar hen (Table 2.1). A possible explanation for this could be related to the hens’ fear of a novel environment. Although hens were familiarised to the runway apparatus, it is possible that the runway tests still evoked fear, as the hens were taken away from their home cage and placed in a novel environment. The presence of a conspecific in a novel environment can aid in the reduction of fear in chickens (Jones and Merry, 1988) and Jones (1984) found that the fear-related responses of chicks in an open-field test were reduced when the chick was in the company of a familiar conspecific rather than an unfamiliar chick. This suggests that in a fear-provoking situation, such as a novel environment, birds that are more fearful may choose to spend more time near a familiar bird rather than an unfamiliar bird compared to hens that are less fearful. This could imply that hens with a higher familiarity score from the runway test may have found the situation more fear-provoking in comparison to birds which spent more time near an unfamiliar bird.

Although the hens were also familiarised to the Y-maze, the novel Y-maze environment may also have evoked fear in the birds. An unfamiliar or novel environment, like that of the Y-maze apparatus, may potentially inhibit or reduce the number of dustbathing bouts performed compared to that observed in a familiar environment. Olsson and Keeling (2005) suggested that a bird is unlikely to commence a dustbathing bout unless it feels secure, as in the wild a bird is vulnerable to predators while dustbathing. Interestingly, dustbathing activity (both in terms of occurrence and duration) was found to have a negative relationship with hen familiarity scores (Figure 2.3 and 2.4). If we accept that the hens that had a higher familiarity score were hens that were more fearful in the novel situation, this may help explain why these birds also dustbathed less in the Y-maze. This relationship between
fear and dustbathing has also been observed in Japanese quail (*Coturnix coturnix japonica*). Gerken *et al.*, (1988) found that quail from a genetic line selected for high dustbathing, also displayed less fear-related responses compared to quail from a low dustbathing line. Similarly, Marin *et al.* (2001) found sociality (i.e. the motivation to be near a conspecific, as measured by the time to traverse a runway to another animal) was positively associated with stress: ‘stressed’ chicks, those which had been exposed to a crush cage for five minutes, one hour before testing, were faster to leave the start box and spent more time near a stimulus bird in comparison to ‘non-stressed’ controls. These results were more pronounced when a familiar chick was used as the stimulus bird compared to an unfamiliar. Therefore, it is possible that fear of the novel environment could have played a role in the hens’ preference to be near a familiar bird in the runway and also on the dustbathing activity in the Y-maze, however, as fear responses were not specifically tested, this cannot be confirmed.

In the Y-maze preference tests, hens in the Intermediate treatment tended to choose peat moss less often compared to the other treatments and were also slower to move through the Y-maze to make their choice. The speed of movement through a Y-maze and other tests in which an animal must move to a resource can be equated to motivation (e.g. Petherick *et al.*, 1992). An animal highly motivated to access a resource would not only choose the resource more often but would also move faster in comparison to an animal with low motivation. This implies that birds in the Intermediate treatment had reduced motivation to gain access to the resource.

Reduced motivation in the Intermediate treatment hens may have been a result of the 20-minute peat moss access time in the Y-maze, as this is less than the reported mean dustbathing bout duration (27 minutes – Vestergaard, 1982). This also explains why hens in the Intermediate treatment had significantly more dustbathing bouts interrupted in the Y-maze compared to hens in the Long treatment (Table 2.1). Mason *et al.* (1998) suggested that repeated interruption of an activity might devalue a resource, particularly if behavioural satiety is reached after only after a certain period. One such example is dustbathing, as this series of behaviours requires a certain period of time to reach a functional result (Hutson, 1984). Repeated dustbathing bout interruption may have caused the Intermediate treatment hens to experience frustration. Frustration has been defined as “an aversive state that results from non-reward, reduced
reward or delayed reward in the presence of a history of reward” (Amsel, 1992). For Intermediate treatment hens, the history of reward would be the ad libitum peat moss in the home cage and these hens would have experienced reduced reward in the Y-maze, as they were interrupted prior to completion of the dustbathing bout. Previous studies have also proposed that dustbathing bout interruption has affected hen motivation. In an experiment using operant conditioning to access peat moss, Widowski and Duncan (2000) suggested that the interruption of dustbathing bouts might have negatively affected hens’ motivation. Moreover, Petherick et al. (1990b) suggested that “it might be quite aversive to birds to be interrupted whilst dustbathing”. Olsson and Keeling (2005) also proposed that the interruption of a dustbathing bout may be aversive to the dustbathing hen. Therefore, the Intermediate hens may have experienced frustration and aversion to the repeated interruption of dustbathing bouts. Intuitively, and has been demonstrated with other species (e.g. sheep, Rushen 1986), it might be expected that the hens would avoid the place that induced these feelings (the Y-maze arm), and show reduced motivation to access the peat.

Interestingly, birds in the Short treatment did not show these indicators of dust devaluation (i.e. reduced speed of movement and tendency to choose dust less). Short treatment birds had all dustbathing bouts interrupted. The difference between the Short and Intermediate treatments is the point in the dustbathing bout where the interruption occurred. Short treatment hens would have been interrupted at the very beginning of the bout (within 2 minutes), whereas Intermediate hens would have been interrupted further into the bout. Perhaps being interrupted right at the beginning of the dustbathing bout is less frustrating and aversive compared to being interrupted further on into the bout because the hen is not fully engaged in the behaviour at the start.

The peat moss provided in the Y-maze proved to be an attractive resource to test hens, as 14 out of the 15 test hens chose it significantly more often than what would be expected by chance. The preference test trials were conducted every second day during the late morning to early/mid afternoon. This interval between test trials and the time of testing was chosen to coincide with the peak occurrence of dustbathing activity in laying hens (Vestergaard, 1982) and therefore, presumed peak dustbathing motivation. Additionally, peat moss has previously been found to be a favoured dustbathing
substrate of laying hens (Petherick and Duncan, 1989; de Jong et al., 2007). Therefore, it may not be surprising that hens overwhelmingly chose peat moss.

However, hens are highly social animals and as the hens were deprived of visual social contact throughout the training and testing phases, it is somewhat surprising that hens had such a low preference for social contact in the Y-maze. Social deprivation in the home cages was imposed by placing opaque rubber partitions between cages. This meant that, although being visually isolated, hens still had auditory and olfactory contact with other birds. Also, while in the start box of the Y-maze, hens could see both Y-maze arms. Perhaps being able to see that there was another hen in the Y-maze together with the sounds and smells of other hens while in the home cage was sufficient social contact for the test hens, thus, leading to the low preference for social contact in the Y-maze.

Interestingly, the results show there was individual variation in terms of dustbathing behaviour in the Y-maze. When peat moss was chosen, some hens chose to dustbathe on every trial, whereas other hens failed to commence a dustbathing bout in the Y-maze (Figure 2.6). This variation was observed in all treatments and, therefore, seems independent of resource quantity. This type of individual variation in peat moss utilisation of hens was also observed in the study by Petherick et al. (1990b) who suggested that access to peat moss “switches on” dustbathing for only some hens, while that was not the case for other individuals. Similarly, recent research by Hemsworth et al. (2009) has found that pigs have shown individual variation in their choice behaviour, with some individuals consistently preferring social contact to food and other individuals consistently preferring food to social contact. In the present experiment, there was variation between hens in whether they dustbathed or foraged when provided with peat moss in the home cage and this variation was consistent for hens peat interaction in the Y-maze. If differences between individuals are found, this may require further research examining genetic and/or experiential effects (Hemsworth et al., 2009). If individual differences are confirmed, this may imply that foraging and dustbathing opportunities differ in their importance to different individuals and thus affect the welfare of animals differently.

Another explanation as to why some hens did not dustbathe in the Y-maze may be that these hens performed sham dustbathing in their home cages during the testing period. It has been proposed that the performance of a sham (or vacuum) behaviour may be an adequate substitute for the real behaviour (e.g. Hughes, 1980 cited in
Lindberg and Nicol, 1997). Therefore, hens that sham dustbathed in the home cage prior to being placed in the Y-maze may have had low motivation to dustbathe. Observations of hens in the home cages were not taken during the testing period, so whether or not sham dustbathing occurred is unknown. However, it has been suggested that hens that have litter experience require a relatively long time to develop sham dustbathing (Hogan, 1994). For example, Vestergaard et al. (1997) deprived litter-experienced hens of litter and found that it took 20 days for hens to show sham dustbathing behaviour. In the present experiment, hens were deprived of peat moss in the home cage for a total of 26 days during training and testing and therefore, could have had enough time to develop sham dustbathing. More recent studies have, however, obtained results that suggest that sham dustbathing is not satisfying or perceived as a real dustbath by hens (Lindberg, 1999; Olsson et al., 2002b; Wichman and Keeling, 2008). This would suggest that, even if hens did sham dustbathe during the testing period, they would still be motivated to dustbathe when given access to peat in the Y-maze.

The social context of a preference test may affect the motivation of an animal. For example, Pedersen et al. (2002) reported that pigs required to perform an operant conditioning task to obtain food were more willing to perform the task when in the presence of another pig compared to when in isolation, making the food reward essentially ‘more valuable’ when in the presence of another pig. Similarly, Sherwin (2003) found that laboratory mice reduced visits to a running wheel when in the presence of other mice compared to when the mouse was in isolation. In the present experiment, hens were in isolation in the Y-maze when they had peat moss access. Dustbathing behaviour may be socially facilitated (Duncan et al., 1998) and does occur in groups (Vestergaard et al., 1990). Petherick et al. (1993) suggested that hens may not engage in dustbathing in a Y-maze due to the social isolation. Therefore, the social isolation of hens in the Y-maze may have reduced the occurrence of dustbathing behaviour.

Why animals show individual variation in their behaviour may be answered from evolutionary biology. From an evolutionary perspective, in a wild situation, individual variation may aid the survival of a species in times of high variability in the local environment (Koolhaas et al., 2007). Although the laying hen was domesticated from the Red Junglefowl approximately 8000 years ago (Oltenacu, 2005), it must be
remembered that the Red Junglefowl had been evolving for millions of years prior to this, from around 150 million years ago (Cracraft, 1993), so it is likely some wild behavioural traits (e.g. individual variation) may still exist in the domestic chicken today.

If scientists are to use preference tests to assess what an animal finds important and consequently what may be necessary for improved welfare, we must first be assured that we have a rigorous methodology by which to assess animal preferences. Results from this experiment, while having some limitations (e.g. a relatively low number of experimental animals) have revealed that just one factor in the design of a preference test can affect animal motivation and potentially, choice behaviour. Future preference studies should take this into consideration when selecting reward quantities and ensure that there is adequate time allowed for behavioural requirements with the resources offered.
CHAPTER 3

THE EFFECTS ON LAYING HENS OF INTERRUPTING A DUSTBATHING BOUT: CHOICE BEHAVIOUR AND ACUTE PHYSIOLOGICAL STRESS

3.1 INTRODUCTION

It has been suggested that interrupting the interaction of an animal with a resource provided in a choice test can devalue the interaction and in turn, the resource (e.g., Mason et al., 1998). Results reported in Chapter 2 support this suggestion, as hens given an intermediate resource quantity (20 minutes) of peat moss showed signs indicative of reduced motivation when compared to hens given a larger (45 minutes) or smaller (2 minutes) resource quantity. The average dustbathing bout duration of laying hens is reported as 27 minutes (Vestergaard, 1982). However, hens given the intermediate reward of peat moss (20 minutes) had an average bout duration of just under 15 minutes (refer to Table 2.2) because many dustbathing bouts were interrupted prior to completion (approximately 65%, refer to Table 2.2). The repeated interruption mid-way through the dustbathing bout may have caused the hens to experience frustration and aversion to the Y-maze arm. Interestingly, hens in the experiment described in Chapter 2 that received 2 minutes of peat moss access did not show signs indicative of reduced motivation. It is possible that interruption of a dustbathing bout at the very beginning of the dustbathing bout did not lead to frustration, as the hen was not fully engaged in the behaviour.

These results warranted further investigation to determine whether the reduced motivation to access peat moss by hens given an intermediate reward was a result of frustration and aversion due to the repeated interruption of dustbathing mid-way through the bout.

A number of authors have questioned the implications of interrupting animals during a behavioural bout in a preference or motivation test (Mason et al., 1997; Mason et al., 1998; Jensen and Pedersen, 2008). For example, Mason et al. (1997) suggested that the repeated interruption of a behavioural bout directed to a resource
may markedly affect consequent motivation for the resource. Jensen and Pedersen (2008) agreed, and proposed that the reward duration in a motivation test should “be long enough for the animal to perform the target behaviour and to find it rewarding, but … not be so long that the animals do not use the time available with the reward efficiently…” They suggested that for some behavioural patterns, breaking the bout into smaller durations can have negative effects on motivation, while this may not be the case for other behaviours (e.g. feeding).

The hypothesis for this experiment was that interrupting dustbathing mid-way through the bout would be frustrating and aversive to laying hens, which would lead to hens avoiding repeated dustbathing interruption. Furthermore, frustration has been shown to result in an increase in plasma corticosteroids (Dantzer et al., 1980) suggesting that it is acutely stressful. Therefore, to assess the stressfulness of dustbathing bout interruption, the plasma corticosterone response was also examined.

To examine these effects, hens were given a choice between interrupted and uninterrupted dustbathing bouts and their choice behaviour and motivation was examined. Based on the findings presented in Chapter 2, dustbathing interrupted after 15 minutes was compared with an uninterrupted bout. A dustbathing bout interrupted after 15 minutes was also tested against a dustbathing bout interrupted after 2 minutes, again to reflect the findings given in Chapter 2. It was hypothesised that the hens would find the 15-minute interrupted bout frustrating and aversive and would avoid it and/or show reduced motivation to access it compared to both the uninterrupted and 2-minute interrupted dustbathing bouts. In addition, as frustration induces acute stress, it was anticipated that an increase in plasma corticosterone would be seen in the hens in the 15-minute interruption treatment.

3.2 MATERIALS AND METHODS

3.2.1 Animals and housing

Selection of experimental animals

Experimental hens were chosen from a flock of caged laying hens that had previously been utilised in an experiment examining nest box usage in cages. The flock was housed in a pre-experimental facility. A total of 96 laying hens (Hy-Line
Brown laying strain) were housed in cages (1.14 m x 0.50 m x 0.45 m) in either groups of eight or individually. As the hens had not previously had dust experience, a plastic tray (0.46 m x 0.24 m x 0.03 m) was placed in each cage 18 days prior to the commencement of dustbathing observations. Each tray was refilled daily with peat moss (TE-EM Canadian Sphagnum Peat Moss) at approximately 1100 h. The hens were 30 weeks of age when the trays were first placed in their cages.

As this experiment aimed to determine the effects of interrupting a dustbathing bout in a Y-maze apparatus, it was important that experimental birds were highly motivated to dustbathe in the Y-maze. Results from Chapter 2 and Petherick et al. (1990b) indicate that some hens do not dustbathe at all in a Y-maze. Furthermore, during pre-experimental observations described in Chapter 2, it was found that ‘dustbathers’ (birds which dustbathed on >85% of peat-chosen Y-maze trials) showed significantly more dustbathing activity during the hour following the daily refilling of the home cage dustbath compared to hens classified as ‘non-dustbathers’ (birds which dustbathed on <15% of peat-chosen Y-maze trials, Table 2.3). Therefore, in an attempt to choose ‘dustbathers’ for this experiment, hens’ dustbathing activity, following the refilling of the dustbath in the home cage, was observed.

Over five consecutive days, all dustbaths were refilled with peat moss at 1100 h. Immediately following this, hens were observed for dustbathing activity during six scans, once every 10 minutes for 60 minutes. Birds in cages that contained eight birds were leg banded to ensure individuals could be identified.

The number of scans that dustbathing was observed for each bird during the five days were summed and the 20 hens with the most dustbathing observations were chosen as experimental hens. The 16 hens that had the highest number of dustbathing observations of these 20 were utilised as test hens. The remaining hens were kept as spare animals.

*Experimental hen housing*

Selected hens were moved to the experimental facility and housed in individual cages (0.57 m x 0.50 m x 0.48 m). Each cage contained an external feed trough at the front of the cage and 1-2 nipple drinkers at the rear. Hens had *ad libitum* feed (a commercial layer pellet feed) and water in their home cage throughout the
experiment. Each cage also contained a plastic tray identical to that which was in the original cages.

Hens had 14 days to settle into the experimental facility. Each day hens were checked, fed and trays filled with peat moss at approximately 1030 h. The hens were kept at an environmental temperature of about 20°C (mean 19.9°C, range 15.3°C to 26.4°C) and on a light:dark cycle of 16:8 h, with lights coming on at 0500 h.

3.2.2 Pre-experimental handling

To ensure hens were accustomed to being handled, all hens were handled as per Chapter 2. This was conducted immediately following the 14-day settling in period.

3.2.3 Preference testing

Y-maze apparatus

The Y-maze apparatus utilised in Chapter 2 was used in this experiment.

Y-maze familiarisation

The hens were familiarised to the Y-maze once per day over four consecutive days, commencing at 1100 h. In a randomly determined order, each hen was placed in the start box (SB) of the Y-maze with both arm gates open and no resources present. After 10 seconds the SB gate was opened and the hen was allowed to leave. If the hen had not left within 30 seconds she was gently pushed forward (with a flat hand on the tail). The SB gate was closed once she had left the SB. Each hen was given a total of five minutes to freely explore the apparatus once she had left the SB.

Y-maze training

This experiment was conducted over two periods of Y-maze training and testing. In each period, half of the hens (n=8) were allocated to one of two treatments that differed in the choice of dustbathing bout duration available in the arms of the Y-
maze (A or B, table 3.1). Each individual was therefore allocated to each treatment over the two periods.

Table 3.1 Description of Y-maze treatments

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Option 1 in Y-maze</th>
<th>Option 2 in Y-maze</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Treatment A</strong></td>
<td>Dustbathing bout interrupted after 15 minutes</td>
<td>Dustbathing bout interrupted after 2 minutes</td>
</tr>
<tr>
<td><strong>Treatment B</strong></td>
<td>Dustbathing bout interrupted after 15 minutes</td>
<td>Dustbathing bout not interrupted</td>
</tr>
</tbody>
</table>

Hens were trained and tested on alternate days, so as to coincide with peak dustbathing activity (Vestergaard, 1982) and therefore presumed peak dustbathing motivation. The hens’ home cage trays were removed for each of the training and testing periods.

A tray, identical to that in the home cages, was placed in each arm of the Y-maze. Each Y-maze arm was allocated a dustbathing bout duration (either 2 minutes, 15 minutes or uninterrupted), depending on the hen’s treatment (Table 3.1). To assist the birds to learn to associate the Y-maze arm with the dustbathing bout length, a coloured board (black or white) was placed on the far wall in each Y-maze arm, which was visible to the hen when in the start box (Figure 3.1). The side that each coloured board was placed was randomly determined for each hen and remained at the same position, along with the designated dustbathing bout duration, for each individual on each treatment. As increased lighting can encourage dustbathing (Hogan and van Boxel, 1993; Duncan et al., 1998), a lamp with a 40-watt globe was placed directly over the dustbath in each of the Y-maze arms.
Each hen received six training sessions, conducted on alternate days. The training sessions provided hens with equal exposure to both Y-maze arms. In each training session each hen was given two consecutive Y-maze training trials in which access was given to only one Y-maze arm, by closing the gate of the other Y-maze arm. The arm accessible first was alternated in each training session.

For each training session, hens were placed individually in the Y-maze start box (SB), in a randomly determined order. After 10 seconds, the SB gate was opened and the hen was allowed 30 seconds to leave. If she had not left the SB voluntarily in this time, she was gently pushed out (flat hand to the tail). The SB gate was closed once the hen had left the SB. The hen was then given a further 30 seconds to enter the open arm. If she had not, she was gently pushed into the open arm and left there for 15 minutes. During this 15-minute period, the hen was closely observed via real time video footage on a monitor outside the experimental room, which was connected to cameras (black and white digital CCD) mounted directly above each Y-maze arm. If a dustbathing bout commenced (defined as when the hen first rolled on to her side) during this period, the hen was given the time for dustbathing designated for the treatment allocated to her in that arm. If the hen had not commenced a dustbathing bout in the 15-minute period, she was picked up and returned to the SB for the second training trial, for which the other Y-maze arm was available, and the process repeated.
Y-maze testing

Each hen was given eight test trials, once per day on alternate days. Y-maze testing was conducted in a similar manner as training, however, both Y-maze arms were open and the hen was allowed to make a choice between the two arms. The hen was defined as choosing the Y-maze arm when she entered one of the arms by crossing over the ‘choice line’ (position of arm gate, refer Figure 3.1). Once the hen had made a choice, the arm gate of the arm not chosen was closed. Like training, once the hen had moved into one of the Y-maze arms, she was given 15 minutes to commence dustbathing and then the time designated for dustbathing in that arm. If she had not commenced in the 15-minute period, she was returned to her home cage.

For each trial the time to choice and the arm chosen were recorded. In addition, whether the hen commenced a dustbathing bout was recorded and if so, the latency to dustbathe and duration of the dustbathing bout were also recorded. A dustbathing bout was defined as completed when the hen stood up and shook. The latency to commence dustbathing was defined as the time from when the SB gate was lifted to the time the hen commenced dustbathing.

At the completion of the eight trials, hens were given 13 days ‘recovery’ in which the home cage dustbaths were replaced and refilled daily with peat moss. After the recovery period, hens were re-trained and tested in the Y-maze under the alternative treatment (Table 3.1) in an identical manner.

3.2.4 Assessment of plasma corticosterone

Blood samples were taken from all hens on trials 4 and 7 in both periods. An initial sample was obtained from each hen immediately prior to being placed into the start box for the test trial to obtain an estimate of the basal level of plasma corticosterone of each individual. A second sample was collected 15 minutes after the hen had been returned to her cage, so as to coincide with the peak plasma corticosterone response to a stressor (Litten and Cockrem, 2001). Samples were obtained regardless of whether the hen engaged in a dustbathing bout or not.

To obtain a blood sample, birds were placed on a table on their side with feet restrained and wing extended away from the body. Blood was collected via venipuncture of the wing vein using a 23G x 2.5 cm long needle attached to a 1.2-mL
lithium heparin monovette. In all cases, blood samples were obtained in under 2 minutes. The Samples were spun in a centrifuge (6000 rpm for 3 minutes) and plasma poured off into a 2-mL microtube before being frozen and stored at -20°C until corticosterone assays were performed. The plasma corticosterone concentration was determined by use of a commercial enzyme immunoassay kit (Immunodiagnostic Systems Limited).

3.2.5 Statistical analysis

The choice behaviour for dustbathing bout durations and the choice for the left or right side of the maze were compared to chance level (i.e. 50:50) by a Chi-square test.

The proportion of trials the 15-minute bout was chosen (separated into all trials and for trials where dustbathing occurred), the proportion of trials dustbathing occurred and mean time to choice were calculated for each bird in each of the two periods. In calculating average latency to commence a dustbathing bout, a trial latency was imputed to be 900 seconds when a bird was not observed to commence dustbathing (i.e. the period of time (15 minutes) each hen had to commence a dustbathing bout once a Y-maze arm was selected). The proportion of trials the 15-minute bout was chosen and the logarithm of average times were analysed as two treatments, 16 individuals, two period cross-over design analyses of variance. Some measurements (the proportion of trials 15-minute bout selected overall and only when dustbathing occurred) used an indicator of whether the non-15 minute bout (i.e. the 2 minute or unrestricted bout) was allocated to the left or right side of the maze as a covariate. In these cases the lowest stratum intra-block analysis of variance effects of side allocated to the non-15 min bout are presented.

The logarithm of corticosterone levels in prescribed groupings (when the 15-minute dustbathing bout was selected, when the non-15 minute bout selected and when dustbathing occurred) were analysed using a mixed model restricted maximum likelihood (REML) analysis with a random effect for bird and fixed effects for period and treatment. The effects of treatment were examined using Wald F tests.
3.3 RESULTS

3.3.1 Preference testing

In both treatments (A and B), hens did not show a statistically significant preference when all trials were examined (treatments 2 vs. 15 (A) and unrestricted vs. 15 (B), $\chi^2 = 0.640; P = 0.69$ and $\chi^2 = 0.160; P = 0.42$ respectively) or when only trials when dustbathing occurred were examined (treatments A and B, $\chi^2 = 0.640; P = 0.69$ and $\chi^2 = 0.00; P = 1.00$ respectively).

When the two treatments (A and B) were compared, there was no significant difference between the dustbathing bout lengths chosen when all trials were examined (P=0.48) and on trials when dustbathing occurred only (P=0.49) (Table 3.2). Additionally, the time to choice was not significantly different between treatments (P=0.27). The latency to commence a dustbathing bout did not differ between treatments (P=0.45), when only the 15-min bout was chosen (P=0.58), or the other bout (i.e. either 2-min or unrestricted bout) (P=0.66) in both treatments (Table 3.2).

The proportion of times each dustbath was chosen and a dustbathing bout was commenced is shown in Table 3.3
### Table 3.2 Summary of choice behaviour, time to choice and dustbathing data for preference testing.

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Covariate</th>
<th>Transformation</th>
<th>Transformed Mean/Proportion (Back transformed mean/proportion)</th>
<th>Treatment A</th>
<th>Treatment B</th>
<th>SED</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proportion of trials 15 minute chosen (all trials)</td>
<td>Side of non-15 minute bout (left vs. right)</td>
<td>-</td>
<td></td>
<td>0.54</td>
<td>0.48</td>
<td>0.076</td>
<td>0.48</td>
</tr>
<tr>
<td>Proportion of trials 15 minute was chosen (only when dustbathing occurred)</td>
<td>Side of non-15 minute bout (left vs. right)</td>
<td>-</td>
<td></td>
<td>0.56</td>
<td>0.50</td>
<td>0.093</td>
<td>0.49</td>
</tr>
<tr>
<td>Proportion of trials a dustbathing bout was commenced</td>
<td></td>
<td>-</td>
<td></td>
<td>0.76</td>
<td>0.73</td>
<td>0.052</td>
<td>0.66</td>
</tr>
<tr>
<td>Time to choice (seconds)</td>
<td></td>
<td>Log₁₀</td>
<td></td>
<td>0.77(5.8)</td>
<td>0.89(7.8)</td>
<td>0.108</td>
<td>0.27</td>
</tr>
<tr>
<td>Latency to dustbathe (all trials) (seconds)</td>
<td></td>
<td>Log₁₀</td>
<td></td>
<td>2.58(380)</td>
<td>2.61(410)</td>
<td>0.046</td>
<td>0.45</td>
</tr>
<tr>
<td>Latency to dustbathe when 15 minutes was chosen (seconds)</td>
<td></td>
<td>Log₁₀</td>
<td></td>
<td>2.53(340)</td>
<td>2.56(360)</td>
<td>0.054</td>
<td>0.58</td>
</tr>
<tr>
<td>Latency to dustbathe when non 15-minute bout was chosen (seconds)</td>
<td></td>
<td>Log₁₀</td>
<td></td>
<td>2.56(360)</td>
<td>2.59(390)</td>
<td>0.060</td>
<td>0.66</td>
</tr>
</tbody>
</table>
Table 3.3 The number of trials each dustbathing bout was chosen and a dustbathing bout was commenced.

<table>
<thead>
<tr>
<th></th>
<th>Treatment A</th>
<th>Treatment B</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2 minutes</td>
<td>15 minutes</td>
</tr>
<tr>
<td>Number of trials chosen</td>
<td>59</td>
<td>69</td>
</tr>
<tr>
<td>Number of chosen trials a dustbathing bout was commenced</td>
<td>51</td>
<td>52</td>
</tr>
<tr>
<td>Proportion of trials dustbathing was commenced</td>
<td>0.864</td>
<td>0.754</td>
</tr>
</tbody>
</table>

3.3.2 Effect of side of Y-maze

The hens overall choice behaviour is shown in Table 3.4. Overall, hens chose the right side significantly more often than would be expected at chance level ($\chi^2 = 11.560$, $P = 0.0007$).

When the effect of side and choice was examined, hens chose the non-15 minute bout more often when it was placed in the right maze arm compared to the left (Table 3.5).

Table 3.4 Y-maze arm choice by hens given the choice between two dustbaths differing in dustbathing bout duration/interruption.

<table>
<thead>
<tr>
<th></th>
<th>Left</th>
<th>Right</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proportion of trials (number of trials)</td>
<td>0.332 (85)</td>
<td>0.668 (171)</td>
<td>1.00 (256)</td>
</tr>
</tbody>
</table>
Table 3.5  Effect of side of Y-maze allocated to non-15 minute bout on the proportion of trials non-15 minute chosen.

<table>
<thead>
<tr>
<th>Side of non-15 minute bout</th>
<th>SED</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Left</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Right</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proportion of trials non-15 minute bout selected (all trials)</td>
<td>0.32</td>
<td>0.70</td>
</tr>
<tr>
<td>Proportion of trials non-15 minute bout selected (only trials where dustbathing occurred)</td>
<td>0.31</td>
<td>0.76</td>
</tr>
</tbody>
</table>

3.3.3 Plasma corticosterone

When the non-15 minute dustbathing bout was selected, Treatment B (15-minute vs. unrestricted) had a higher corticosterone mean compared to Treatment A (2-minute vs. 15-minute) (P = 0.021) (Table 3.6). However, when the ratio of post-test to pre-test corticosterone was examined, there was no difference between treatments (P = 0.62).

Figure 3.2 shows the ratio of pre-test to post-test corticosterone, separated into the following types of dustbathing bouts; no dustbathing, interrupted after 2 minutes, interrupted after 15 minutes and uninterrupted dustbathing bout.
Table 3.6  Plasma corticosterone concentrations (ng/ml) of hens pre- and post-test and the ratio of pre- and post-test. Pre-test refers to samples obtained immediately prior to the hen placed in the Y-maze, post-test refers to samples obtained 15 minutes after the hen was removed from the maze. Samples were obtained on trials 4 and 7 of each of the two testing periods. P-values in bold indicate significant difference. P-values in italics indicate tendencies.

<table>
<thead>
<tr>
<th>Measurement</th>
<th>Transformation</th>
<th>Transformed Mean</th>
<th>Backtransformed Mean</th>
<th>P-Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Treatment A</td>
<td>Treatment B</td>
<td>SED</td>
</tr>
<tr>
<td>Pre-test corticosterone (overall)</td>
<td>$\log_{10}$</td>
<td>-0.34</td>
<td>-0.25</td>
<td>0.094</td>
</tr>
<tr>
<td>Post-test corticosterone (overall)</td>
<td>$\log_{10}$</td>
<td>-0.32</td>
<td>-0.21</td>
<td>0.068</td>
</tr>
<tr>
<td>Ratio of post-test corticosterone to pre-test</td>
<td>$\log_{10}$</td>
<td>0.01</td>
<td>0.04</td>
<td>0.096</td>
</tr>
<tr>
<td>corticosterone (overall)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-test corticosterone when non-15 min selected</td>
<td>$\log_{10}$</td>
<td>-0.49</td>
<td>-0.21</td>
<td>0.138</td>
</tr>
<tr>
<td>Post-test corticosterone when non-15 min selected</td>
<td>$\log_{10}$</td>
<td>-0.38</td>
<td>-0.11</td>
<td>0.086</td>
</tr>
<tr>
<td>Ratio of post-test corticosterone to pre-test</td>
<td>$\log_{10}$</td>
<td>0.13</td>
<td>0.06</td>
<td>0.126</td>
</tr>
<tr>
<td>corticosterone when non-15 min selected</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-test corticosterone when 15 min selected</td>
<td>$\log_{10}$</td>
<td>-0.36</td>
<td>-0.39</td>
<td>0.076</td>
</tr>
<tr>
<td>Post-test corticosterone when 15 min selected</td>
<td>$\log_{10}$</td>
<td>-0.33</td>
<td>-0.22</td>
<td>0.060</td>
</tr>
<tr>
<td>Ratio of post-test corticosterone to pre-test</td>
<td>$\log_{10}$</td>
<td>0.02</td>
<td>0.07</td>
<td>0.046</td>
</tr>
<tr>
<td>corticosterone when 15 min selected</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-test corticosterone when dustbathing</td>
<td>$\log_{10}$</td>
<td>-0.35</td>
<td>-0.36</td>
<td>0.068</td>
</tr>
<tr>
<td>occurred</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Post-test corticosterone when dustbathing</td>
<td>$\log_{10}$</td>
<td>-0.40</td>
<td>-0.27</td>
<td>0.060</td>
</tr>
<tr>
<td>occurred</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ratio of post-test corticosterone to pre-test</td>
<td>$\log_{10}$</td>
<td>-0.05</td>
<td>0.09</td>
<td>0.101</td>
</tr>
<tr>
<td>corticosterone when dustbathing occurred</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
3.2 Figure 3.2 Dot histogram of the ratio of pre-test to post-test plasma corticosterone. No DB = hens did not dustbathe (n=16); 2 min = hens were interrupted 2 minutes into the dustbathing bout (n=9); 15 min = hens were interrupted 15 minutes into the dustbathing bout (n=27); Unint = Hens were not interrupted and were allowed to complete their dustbathing bout (n=11). Samples were obtained from all hens on trials 4 and 7 of each period.

3.4 DISCUSSION

Hens did not show a preference in either Y-maze treatment, which contradicts the hypothesis based on results obtained from work described in Chapter 2. Those results suggested that a dustbathing bout interrupted midway through the bout may cause frustration and reduce the motivation of hens to subsequently gain access to dust. There are two possible reasons to explain this lack of preference. Firstly, the hens were unable to learn the association between the Y-maze arm, visual cue and length of dustbathing bout. Or secondly, the interruption of a dustbathing bout did not cause frustration to hen.

This experiment required hens to learn and remember the association between Y-maze arm and dustbathing bout duration/interruption. A visual cue was also used to aid learning. Hens had to make an initial choice between two identical trays of peat moss coupled with a visual cue, which only differed when and if a dustbathing bout
was commenced. Whether an animal can choose based on the long-term consequences of a choice versus the short-term gain has long been argued (e.g. Duncan, 1978; van Rooijen, 1982). However, recent research by Abeyesinghe et al. (2005) found that hens were willing to forgo a small short-term (food) reward for a larger long-term reward (i.e. they were willing to wait longer), provided that the large reward was adequately valuable (i.e. the amount of food reward was large).

It was hypothesised that the repeated interruption of a dustbathing bout would be frustrating to hens and, if so, they would learn to avoid it. Rushen (1996), using examples for sheep, states that “animals can learn to predict, from certain cues or signals, how they are to be handled, and then show some aversion to these signals” (e.g. Rushen, 1986; Rushen and Congdon, 1986). By this same principle, animals may then be able to learn to associate a cue with other aversive or frustrating situations. Therefore, if interrupting a dustbathing bout was very aversive or unpleasant to the hen, it would be expected that she would avoid it by choosing the other Y-maze arm. However, this was not the case, leading to the suggestion that interrupting a dustbathing bout did not cause hens to become frustrated as thought from the findings of the experiment described in Chapter 2.

Evidence that hens did not become frustrated as a result of dustbathing bout interruption is supported by the fact that the ratio of pre-test to post-test plasma corticosterone concentrations was not different between interrupted bouts, uninterrupted bouts and hens that did not dustbathe (Figure 3.2). Samples from hens that did not dustbathe (the ‘no dustbathing’ group) were collected to reflect the effects of handling and being placed into the Y-maze, without dustbathing. One limitation with the ‘no dustbathing’ group samples is that, like the other dustbathing groups, blood samples were obtained 15 minutes after the hen had been returned to the home cage. This meant for hens in the other groups (i.e. interrupted after 2 or 15 minutes, or uninterrupted), the second sample would be collected 15 minutes after being interrupted so as to coincide with peak plasma corticosterone response to a perceived stressor (Litten and Cockrem, 2001). However, for hens that did not dustbathe, the second sample was collected essentially 30 minutes after initial handling and introduction to the Y maze (i.e. the 15-minute period available in the Y-maze in which to commence dustbathing and then an additional 15 minutes in the home cage after being returned). Therefore, the peak corticosterone level for hens in the ‘no
dustbathing’ group may have been missed and the value obtained may not have been indicative of the true physiological acute stress response.

When the non-15 minute bout was selected, hens in treatment B (uninterrupted bout selected) had a tendency to have a higher plasma corticosterone level pre-test (i.e. immediately prior to being placed in the Y-maze) and also a significantly higher corticosterone level 15 minutes after being returned to the home cage compared to treatment A. Moreover, treatment B hens also had a tendency to have a higher plasma corticosterone level post-test when dustbathing occurred. Interestingly, the ratio of pre-test to post-test corticosterone did not differ between treatments. An increase in plasma corticosterone is indicative of an acute physiological stress response (e.g. Matteri et al., 2000) and, as seen with other forms of activity and arousal such as copulation (Szechtmann et al., 1974), an increase in corticosterone may also occur with dustbathing. It may therefore be possible that hens allocated to treatment B had an increase in corticosterone due to a state of arousal in the Y-maze. When these hens were picked up out of their home cage, they may also have been in a state of arousal as an anticipatory response to being placed in the Y-maze. The physiological responses of hens following both dustbathing and interrupted dustbathing require more comprehensive examination.

An interesting finding from this experiment was that hens showed a side preference, with a side bias to the right (Table 3.3). Side preferences in hens in Y-maze preference studies are rare in the scientific literature, however, Petherick et al. (1993b) reported that hens made more ‘correct’ choices (i.e. selected the resource of which they were deprived) more often on the right than the left of the maze. Results from the present experiment are similar to those reported in Petherick et al. (1993b) as hens selected the non-15 minute bout more often in the right maze arm compared to the left (Table 3.5). When hens are preference tested, there are usually two different resources presented. Therefore, if a hen does have a preference for one particular side to the other, this may be masked by the distraction of, or motivation for the resources on offer. However, in this experiment each arm contained the same item. Therefore, if hens were unable to differentiate between the dustbathing bout durations, they would essentially make a free choice and this may have led to the display of an underlying side preference. This may be an important factor to explore in preference tests, so as to ensure animals are not making choices caused by the position of the resource.
Although rare in chickens, numerous studies have observed individual dairy cows to have a side preference when entering a two-sided dairy, which may remain constant over a long period of time (Gadbury, 1975; Hopster et al., 1998; Paranhos da Costa and Broom, 2001; Prelle et al., 2004). Although the cause of dairy cattle side preferences is unknown, it has been suggested to be a result of previous experience (Grandin et al., 1994; Hosoi et al., 1995), neurological development (Tanner, 1994) or social behaviour (Hopster et al., 1998).

Side preferences and other bodily side bias, such as ‘handedness’ or ‘footedness’, are related to the lateralization of the brain. Brain lateralization, which is where the left and right hemispheres of the brain differ in terms of structure or function (Rogers, 2000), has been found in a range of lower and higher-order vertebrate species (Rogers, 2000). For example, a number of parrot species have been found to prefer one foot to the other for holding and manipulating food (reviewed by Harris, 1989). The domestic chick has also been found to have a preferred foot to initiate scratching while foraging (Rogers and Workman, 1993; Tommasi and Vallortigara, 1999).

Research into lateralization of the brain in vertebrates has found that the two hemispheres differ in the way they process information and behaviours they control (Rogers, 2009). It has been found that the right brain hemisphere (and therefore left eye) deals with potential threats and is the hemisphere that controls the expression of emotion. For example, adult hens respond to aerial predator alarm calls by looking up with their left eye (Evans et al., 1993). Laboratory rats show increased exploratory behaviour (and thus less fear) in an open field when they have received lesions to the right brain hemisphere compared to the left hemisphere (e.g. Pearlson and Robinson, 1982). Furthermore, domestic dogs are more responsive to threatening stimuli when presented on the left compared to the right (Siniscalchi et al., 2010). Conversely, the left brain-right eye system deals with complex tasks and is the dominant eye for visual discrimination tasks (e.g. Gunston and Gunston, 1984; Mench and Andrew, 1986). For example, when chicks were required to peck for grain on a background of grain-sized pebbles, chicks that used only the right eye performed better (in terms of pecking less at the pebbles and more at the grain) compared to chicks which could only use their left eye (Mench and Andrew, 1986). These differences in eye use in animals could lead to side bias in the Y-maze. For example, as hens showed a
preference to the right, perhaps they favoured their right eye to evaluate the maze and consequently also moved into the right maze arm.

Hens in this experiment were given six training sessions conducted on alternate days. This was similar, but longer than, the training phase in the experiment described in Chapter 2 where hens were successful at making choices in the Y-maze. The proportion of trials that dustbathing was commenced was recorded during training, however, this was not related to the hens’ choice. Perhaps future studies that use a similarly complex task may consider a different approach to training. For example, experimental hens may be selected as hens which reach a predetermined criterion during the training session to increase the probability that experimental hens will have learnt the task.

Overall, the lack of preference for uninterrupted dustbathing suggests that the interruption of a dustbathing bout does not cause hens to become frustrated as predicted. This is supported by the fact the plasma corticosterone responses did not differ between uninterrupted and interrupted bouts. However, it is possible that the hens were unable to learn the association between Y-maze arm and dustbathing bout duration/interruption. Further research investigating hen learning ability may be useful in interpreting these results.
CHAPTER 4

AN INVESTIGATION INTO THE LEARNING CAPABILITY OF LAYING HENS IN A Y-MAZE TASK

4.1 INTRODUCTION

Results from the experiment described in Chapter 3 revealed that hens did not show a preference for an uninterrupted dustbathing bout. These results were somewhat unexpected as results from the experiment described in Chapter 2 suggested that the interruption of dustbathing approximately 15 minutes into the dustbathing bout may be cause frustration and reduced preference for this situation. One of the possibilities for the results obtained in Chapter 3 is that hens were not able to learn the association between Y-maze arm, visual cue and reward.

This experiment examined the learning capability of laying hens in a simple Y-maze task. Hens were presented with a tray of peat moss (a reward) versus an empty tray, with hens being unable to see the tray contents until after they had made a choice. These trays were also associated with a visual cue, in a similar manner to that in the experiment described in Chapter 3.

Petherick et al. (1990b) conducted a similar experiment where hens had to learn the association between coloured doorway and access to a peat-reward in a Y-maze. The main difference between the Petherick et al. (1990b) study and the present experiment is that the hens in this experiment were presented with the trays remaining in the same Y-maze arm for each hen throughout the training and testing period. Therefore, hens in this experiment had the location of the trays in addition to a visual cue to help them learn the position of the reward.
4.2 MATERIALS AND METHODS

4.2.1 Animals and housing

Twenty beak-trimmed hens (Brown Hy-sex Ingham laying strain) were obtained from a commercial farm. The hens were 70 weeks of age when they were obtained and had only experienced living in conventional cages under commercial conditions. Hens were transported to the experimental facility, where they were randomly allocated into individual cages (0.57 m x 0.50 m x 0.48 m). Each cage had an exterior feed trough, one to two nipple drinkers and a plastic tray (0.46 m x 0.24 m x 0.03 m). The tray was filled daily with approximately 400mL peat moss (Canadian TE-EM sphagnum peat moss). Hens were previously fed a commercial mash feed, however, over the course of seven days, hens were weaned onto a layer pellet feed (Barastoc Golden Yolk layer pellets) which they were fed ad libitum for the rest of the experimental period. Hens were transferred to a pellet feed as it is possible that pellet feed would be utilised less as a dustbathing substrate in comparison to a mash feed, as the mash resembles the structure of peat moss.

The hens had four weeks to settle into the facility prior to the commencement of the experiment. Each morning, hens were checked, fed, drinkers checked and trays refilled with peat moss at approximately 1030 h. The facility was maintained at an environmental temperature of about 21°C (mean 20.7°C, range 16.0°C to 24.5°C) and a light:dark cycle of 16:8 h, with lights coming on at 0500 h.

4.2.2 Pre-experimental handling

To accustom hens to being handled, hens were handled in an identical manner as described in Chapter 2 and 3.

4.2.3 Preference testing

Y-maze apparatus

The Y-maze apparatus utilised in Chapter 2 and 3 was used in this experiment.
**Y-maze familiarisation**

Sixteen hens were randomly chosen to be experimental animals for the Y-maze task. These 16 hens were firstly familiarised to the Y-maze apparatus. Each hen was placed in the Y-maze for five minutes once per day on four consecutive days. The Y-maze was empty and both Y-maze arm gates were open to allow the hen to freely explore the apparatus.

In a randomly determined order, each hen was placed in the Y-maze start box (SB) for 10 seconds before the SB gate was opened. Once the SB gate was opened, the hen had 30 seconds to leave the SB to enter the choice area. If she had not left in this time, she was gently pushed out of the SB and the SB gate closed behind her. The hen remained in the Y-maze for five minutes from when the SB gate was opened.

**Y-maze training**

A tray was placed in each of the Y-maze arms for each hen, however, only one of these dustbaths was filled with peat moss. The trays that were placed in each Y-maze arm were identical to the those that had been placed in the hens’ home cages, however, an additional ‘lip’ was placed on one of the long sides of the bath to prevent hens seeing the contents of the dustbath prior to entering the Y-maze arm. This ‘lip’ was composed of opaque rubber and was attached to the dustbath with gaffer tape. The lip was 0.11 m in height, which was calculated as being greater than the minimum height that prevented hens seeing the tray contents from the choice area in the Y-maze (refer Appendix I).

Each hen was presented with a tray in each Y-maze arm. One of the trays contained peat moss (PT) and the other was empty (ET). Half of the hens received PT in the right maze arm, while the other half received PT in the left. Each hen also had a visual cue (a black or white board) allocated to each Y-maze arm. The allocation of visual cue to the PT and ET was balanced. The arm in which the PT was allocated and the position of the visual cue remained constant for each hen throughout the training and testing period.

Hens received six training sessions, which were conducted on alternate days, so as to coincide with the occurrence of dustbathing behaviour (Vestergaard, 1982) and, therefore, presumed peak dustbathing motivation. Home cage dustbaths were
removed on the day prior to the first training session and were not returned until after completion of the experiment. In each training session, each hen received two consecutive Y-maze trials in which only one Y-maze arm was available at a time, essentially ‘forcing’ the bird to move into one arm at a time. The order of hens for training was randomised each training session, and the Y-maze arm available first was randomly determined for each hen on the first trial and then changed on the next training trial.

For each training session, each hen was placed in the start box (SB) for 10 seconds before the SB gate was opened. The hen had 30 seconds to leave the SB. If she had not left the SB in 30 seconds, she was gently pushed forward. Once the hen had left the SB, the SB gate was closed. Once in the choice area, the hen had a further 30 seconds to enter the available Y-maze arm. If she had not moved into the arm in this time, she was gently pushed towards the arm until she had entered (i.e. crossed the ‘choice line’ which was the position of the opened arm gate). Once in the arm, the hen remained there for two minutes. (Two minutes was chosen as the amount of time with the selected resource based on findings from the experiment described in Chapter 2). The hen was then returned to the SB for the second trial in which the alternate arm was available.

**Y-maze testing**

Y-maze testing was identical to training, however, both Y-maze arms were open and once the hen had left the SB, the hen was able to move into the Y-maze arm of her choice. Once the hen had moved into a Y-maze arm, the gate of the other arm was closed and the hen remained in the Y-maze for two minutes before being returned to her home cage.

Hens were tested in a randomly determined order for 8 test trials (one trial per bird per day) that were conducted on alternate days. The following data were recorded: hen choice (including resource (PT or ET) and the associated colour (black or white) and side (left or right)), time to choice and, if PT was chosen the nature of the dust interaction (no interaction, foraging behaviour (pecking and scratching the dust) or dustbathing). A dustbathing bout was defined as commencing when the first vertical wing shake occurred (no dustbathing bouts were completed in the two minutes that hens had access with their chosen dustbath).
4.2.4 Statistical analysis

The overall proportion of test trials the PT was chosen was compared to what would be expected at chance level (i.e. 50:50) using a Chi-square test.

The choice behaviour and time to choice variables (latency to leave start box, time to choice (all trials) and time to choice (when peat moss chosen only)) were compared between birds provided with the peat tray on the left and right by a Student’s t-test.

4.3 RESULTS

Three hens (hen number 4, 8 and 13) were excluded from the study, as they did not move voluntarily in the Y-maze during the training phase. Of these three excluded birds, two hens (numbers 4 and 8) had peat moss presented in the left Y-maze arm and the other hen (number 13) had peat moss presented in the right Y-maze arm.

Hens chose the peat-filled tray on 93% of test trials (Table 4.1). This is significantly more often than what would be expected by chance (i.e. 50:50) ($\chi^2 = 73.960, P < 0.001$). Furthermore, nine of the hens selected the dustbath that contained the peat moss on every test trial. The sequence of choice for the four hens that did not select the peat moss on every test trial is shown in Table 4.2.

The side in which the peat tray was placed had no effect on the choice behaviour ($P = 0.347$), latency to leave the start box ($P = 0.435$), time to choice (all trials) ($P = 0.749$) or time to choice when peat moss was chosen ($P = 0.707$) (Table 4.3).
Table 4.1  Individual hen choice behaviour, time to choice and occurrence of dustbathing. Birds number 4, 8 and 13 were excluded from the experiment as they failed to move voluntarily in the Y-maze.

<table>
<thead>
<tr>
<th>Bird number</th>
<th>Mean time to choice (seconds)</th>
<th>Proportion trials peat-filled tray chosen</th>
<th>Proportion of dust-chosen trials dustbathing was commenced</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>5.08</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>2</td>
<td>1.90</td>
<td>1.00</td>
<td>0.875</td>
</tr>
<tr>
<td>3</td>
<td>3.35</td>
<td>1.00</td>
<td>0.75</td>
</tr>
<tr>
<td>5</td>
<td>2.16</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>6</td>
<td>4.86</td>
<td>0.625</td>
<td>1.00</td>
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<tr>
<td>7</td>
<td>2.22</td>
<td>1.00</td>
<td>1.00</td>
</tr>
<tr>
<td>9</td>
<td>2.55</td>
<td>1.00</td>
<td>0.875</td>
</tr>
<tr>
<td>10</td>
<td>1.79</td>
<td>1.00</td>
<td>1.00</td>
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<tr>
<td>11</td>
<td>2.60</td>
<td>0.75</td>
<td>0.50</td>
</tr>
<tr>
<td>12</td>
<td>4.16</td>
<td>0.875</td>
<td>0.875</td>
</tr>
<tr>
<td>14</td>
<td>2.46</td>
<td>0.875</td>
<td>0.50</td>
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<tr>
<td>15</td>
<td>1.56</td>
<td>1.00</td>
<td>1.00</td>
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<tr>
<td>16</td>
<td>2.60</td>
<td>1.00</td>
<td>0.75</td>
</tr>
<tr>
<td>Mean</td>
<td>2.87</td>
<td>0.93</td>
<td>0.85</td>
</tr>
<tr>
<td>Standard Deviation</td>
<td>1.11</td>
<td>0.12</td>
<td>0.18</td>
</tr>
</tbody>
</table>
Table 4.2  Sequence of choice of hens that did not select dust on every trial. PT = peat-filled tray; ET = empty tray

<table>
<thead>
<tr>
<th>Bird number</th>
<th>Side of PT</th>
<th>Test trial number</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>Left</td>
<td>ET   ET   PT    ET   PT   PT   PT   PT</td>
</tr>
<tr>
<td>11</td>
<td>Right</td>
<td>ET   PT   ET    PT   PT   PT   PT   PT</td>
</tr>
<tr>
<td>12</td>
<td>Right</td>
<td>PT   PT   PT    PT   PT   PT   PT   ET</td>
</tr>
<tr>
<td>14</td>
<td>Left</td>
<td>PT   ET   ET    PT   PT   PT   PT   PT</td>
</tr>
</tbody>
</table>

Table 4.3  Effect of side of Y-maze in which the peat moss tray was placed on the choice behaviour and time to choice of hens.

<table>
<thead>
<tr>
<th>Side in which peat moss was placed in Y-maze</th>
<th>SED</th>
<th>t-value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Left</td>
<td>0.916</td>
<td>0.946</td>
<td>0.0179</td>
</tr>
<tr>
<td>Right</td>
<td>0.0179</td>
<td>0.417</td>
<td>0.347</td>
</tr>
<tr>
<td>Proportion of trials in which peat moss was chosen</td>
<td>2.965</td>
<td>3.291</td>
<td>2.008</td>
</tr>
<tr>
<td>Latency to leave start box (seconds)</td>
<td>0.167</td>
<td>0.435</td>
<td></td>
</tr>
<tr>
<td>Time to choice (all trials) (seconds)</td>
<td>0.6804</td>
<td>0.691</td>
<td>0.749</td>
</tr>
<tr>
<td>Time to choice (only when peat moss was chosen) (seconds)</td>
<td>0.7134</td>
<td>0.575</td>
<td>0.707</td>
</tr>
</tbody>
</table>

4.4  DISCUSSION

Hens were clearly able to learn the association between Y-maze arm, visual cue and the presence of peat moss, as hens chose peat moss on significantly more trials than what would be expected at random. Moreover, nine of the 13 test hens selected the peat-filled tray on every test trial. Of the four hens that did not select dust on every trial, three hens (number 6, 11 and 14) chose the peat moss filled tray in the final four test trials (Table 4.2), which may indicate that these hens required more trials to learn the location of the dust reward.

The non-exclusive choice by hens could also be caused by other factors. For example, the hens may select the empty tray to check if it has changed since the previous visit (Arnold, 2005). Alternatively, animals may have to sample each arm to gain information to make a well-informed choice or the animal may misidentify the resource (Nicol, 1986).
In a similar study, Petherick et al. (1990b) found that unless the test trials were massed (i.e. several trials run in quick succession), hens were unable to learn the association between a colour cue and access to peat moss. The main difference between that experiment and the present one is that, in this experiment the tray containing peat moss always remained in the same position, whereas the dust tray in the Petherick et al. (1990b) study was randomly switched (together with the associated visual cue). Therefore, in the current experiment hens probably relied on the position of the peat-filled tray as well as the visual cue. This may imply that position or location of a dustbathing substrate may be more important for hens in learning than a visual cue. As the sight of a dusty substrate may initiate dustbathing behaviour (Petherick et al., 1995), it is possible that free-ranging or feral chickens dustbathe in a similar location (i.e. an area where a dusty substrate is found) in their home range. If so, the location of a dustbathing substrate may also be important to domestic hens.

Although the hens in this experiment were successful at learning the task, it is important to note that the task in this experiment was considerably simpler than that described in Chapter 3. This is because the hens in the experiment described in Chapter 3 had to make a choice between two visually identical trays of peat moss, which differed only after a certain amount of time once dustbathing was commenced. In contrast, in the current experiment, on making a choice hens would get immediate feedback and would know if peat moss was present or not. This implies that the task in the experiment described in Chapter 3 was too complex for the hens. Therefore, the results obtained in Chapter 3 may not been indicative of the hens’ actual preferences for dustbathing bout interruption.

Results from the experiment described in Chapter 3 found that hens had a side bias to the right. Findings from the current experiment found no such result (Table 4.3). A crucial difference between the experiment described in Chapter 3 and the present is that hens were provided with two peat-filled trays in Chapter 3, whereas only one arm contained a peat-filled tray (versus an empty tray) in this experiment. Therefore, perhaps when faced with two similarly attractive resources (i.e. two peat-filled trays), the hens’ side bias is shown. Conversely, when hens are faced with one attractive resource (peat-filled tray) versus a less attractive resource (an empty tray) their side bias is not shown due to the hens’ strong preference for one resource to another.
Three hens were excluded from this experiment as they failed to move voluntarily in the Y-maze. No other birds in the experiments described in previous chapters have had to be excluded, as all previous hens voluntarily moved through the maze. A key difference between the flock of hens used in the present experiment is that they were previously housed at a commercial farm. It is possible that these three birds may have experienced stress or fear that made them unwilling to move in a novel environment.

Overall, this experiment showed that hens are able to learn and remember the position of a reward in a Y-maze although they could not see it when they made their choice.
CHAPTER 5

THE EFFECTS OF THE INTERVAL BETWEEN TESTS AND QUALITY OF RESOURCE ON THE CHOICE BEHAVIOUR OF LAYING HENS FOR A DUST SUBSTRATE AND SOCIAL CONTACT

5.1 INTRODUCTION

The interval between tests in the Y-maze may alter the motivation of an animal. In most Y-maze studies, animals are deprived of the resources of interest in their home cage and only have access to the resource in the test. Therefore, the interval between tests determines the duration of resource deprivation. The period of deprivation may influence animal motivation. For example, hens deprived of food exhibit more exploratory behaviour (Nicol and Guilford, 1991) and are faster to run an alleyway (Petherick et al., 1992) and a Y-maze (Laine et al., 2007) to a food reward, compared to hens not deprived. This indicates that the deprivation of feed increases hen motivation to access feed. Likewise, Nicol and Guilford (1991) also reported that hens deprived of peat moss showed increased exploratory behaviour compared to those not deprived, indicating deprived hens were more motivated to access the peat moss. Therefore, if the period of deprivation impacts the motivation of animals, the interval of testing may be a potentially important factor in the design of animal preference tests.

The interval between tests may be a particularly important factor if behavioural patterns related to the resources under investigation have known temporal rhythms of occurrence. One such example is dustbathing behaviour. The motivation to dustbathe appears to build up over time and laying hens perform, on average, one, 27-minute dustbathing bout every second day, with peak dustbathing activity around midday to early afternoon (Vestergaard, 1982; Vestergaard et al., 1990; Hogan and van Boxel, 1993). This is suggestive of an endogenous circadian rhythm of motivation. In addition, following a period of dustbathing deprivation, hens have been found to reduce the latency to dustbathe and increase the dustbathing bout duration.
when dustbathing is next allowed (Vestergaard, 1982), conforming to the Lorenzian ‘psychohydraulic’ model of motivation (Hogan and van Boxel, 1993).

Like the interval between tests, the quality of a resource on offer may also affect animal motivation. The term quality refers to ‘characteristics with respect to excellence’ (Heinemann Australian Dictionary, 1995). With respect to dustbathing substrates, quality may relate to the effectiveness of the material for its function. The function of dustbathing is reported to be to regulate and distribute feather lipids, thus maintaining plumage (van Liere and Bokma, 1987). Research has found that substrates that are composed of smaller particles, such as sand or peat moss are more efficient at reaching the proximal (plumulous) part of the feathers. They, thus, provide a more effective dustbathing substrate in terms of removing excess feather lipids, compared to substrates composed of larger particles, such as wood shavings (van Liere and Siard, 1991).

This experiment used two dustbathing substrates, sawdust and peat moss. These substrates differed in particle size (see Figure 5.1) and therefore likely quality as a dustbathing substrate. Furthermore, laying hens have been found to prefer specific dust substrates in which to dustbathe. Research has found that peat moss and sand are preferred by hens for dustbathing compared to sawdust or shavings (Petherick and Duncan, 1989; van Liere et al., 1990; Shields et al., 2004; de Jong et al., 2007).

There may also be differences in the quality of social contact. The ‘effectiveness’ of social contact of a particular hen with another hen may be dependant on the relationship between the two hens. Hens prefer to be closer to familiar hens rather than unfamiliar, and subordinate hens to dominant hens (Dawkins, 1982; Bradshaw, 1992; Grigor et al., 1995). Therefore, a familiar and/or subordinate hen may be more effective at providing social contact compared to an unfamiliar and/or dominant.

Social contact is presumably important for laying hens. When isolated, domestic chicks have been found to show behavioural and physiological indicators of stress, however, these stress indictors were reduced when the test chick was placed in the same environment with a conspecific (Jones and Merry, 1988). Moreover, Jones (1984) found that the fear-related responses of a chick in an open field were reduced when the conspecific was a familiar chick compared to an unfamiliar chick. Furthermore, Mills and Faure (1989) reported that when hens were isolated from
familiar conspecifics, the isolated hen showed reduced feeding and increased behaviours that may be related to frustration (movement and preening), implying that social isolation may cause frustration. These findings all indicate that social contact is important for laying hens.

In adult laying hens several behaviours have been found to be socially facilitated with many individuals performing the behaviour in synchrony. Examples include feeding (Hughes, 1971; Webster and Hurnik, 1994), preening (Webster and Hurnik, 1994) and possibly dustbathing (Duncan et al., 1998).

The hypothesis tested in this experiment was that differences in the interval between tests and quality of dustbathing substrate presented in the Y-maze would impact on the motivation, and potentially, the choice behaviour of laying hens. To test this, hens were preference tested in a Y-maze for their choice between social contact and a dustbathing substrate with testing conducted at three intervals. The substrate provided in the Y-maze was either sawdust or peat moss, which differed in their quality as a dustbathing substrate.

5.2 MATERIALS AND METHODS

5.2.1 Animals and housing

Selection of experimental hens.

Experimental hens were selected from a flock of 80 beak-trimmed hens (Brown Hy-line laying strain) sourced from a commercial farm at approximately 22 weeks of age. The hens were transported to a pre-experimental facility where they were housed in 10 cages, each cage (1.14 m x 0.50 m x 0.45 m) housing eight birds. Each cage contained an external feed trough at the front of the cage, three nipple drinkers at the rear and a plastic tray (0.46 m x 0.22 m x 0.03 m). The 10 cages were located in two adjacent rooms, with five cages in each room. The rooms differed in the dust substrate provided in the cages; it was important that the hens experienced only the substrate with which they were to be tested and allocation to different rooms was the best method to ensure there was no cross contamination of substrates. The trays in one of the rooms were filled daily with peat moss (Canadian TE-EM sphagnum peat moss, particle size < 1 mm in width), while the cages in the other
room were filled daily with sawdust (Pollard’s Sawdust Supplies sawdust and
shavings, particle size approximately 3 – 8 mm in width), (refer to Figure 5.1). The
rooms were otherwise maintained in an identical manner. Peat moss and sawdust were
chosen as the two substrates as numerous studies have found that peat moss is highly
preferred over sawdust for dustbathing (Petherick and Duncan, 1989; van Liere and
Siard, 1991; de Jong et al., 2007).

**Figure 5.1** Photograph of dust substrates provided in the experiment. Sawdust is
above the ruler and peat moss is below the ruler. Ruler measurements are in
millimetres. (Photo: S. Laine)

Birds were selected for the experiment at 32 weeks of age on their assessed social
status, defined as either ‘dominant’ or ‘subordinate’. Hens were assessed during 5-
minute direct observations of each cage of hens when feed (Barastoc Golden Yolk
layer pellets) access was re-allowed following overnight feed deprivation (mean of 17
hours deprivation, range of 16.25 to 17.75 hours). Feed deprivation was carried out by covering each cage’s feed trough with three metal covers. When each cage was re-allowed feed access, only one metal cover was removed thus reducing the space of the feed trough (to approximately 0.28 m of feed trough length), which did not provide enough space for all birds in the cage to feed simultaneously. Observations were made each morning, commencing at 1030 hours, over six consecutive days. The order of cage observations was randomised each day. Social interactions associated with the presentation of feed were recorded to assist the assessment of dominant (defined as the hen that pecked another individual at the feed trough or easily regained access to feed) and subordinate (hen was the recipient of a peck at the feed trough, could not easily regain access to feed and/or paced or vocalised while attempting to gain access) behaviour in hens. Individual hens were identified via coloured leg bands. Each time a hen was observed displaying a behaviour designated as either dominant or subordinate, the event was recorded and totals determined. At the end of the observation period, individuals from the same cage that were consistently recorded as dominant and subordinate were chosen as a pair. Twelve pairs of hens (six pairs from each room) were chosen as experimental hens, while a further three pairs were chosen as spares.

**Experimental hen housing**

Selected hens were transported to the experimental facility and housed individually in cages (0.57 m x 0.50 m x 0.48 m). Each cage contained an external feed trough at the front of the cage and one to two nipple drinkers at the rear. Hens had *ad libitum* feed and water in their home cage throughout the experiment. Each cage also contained a plastic tray identical to the tray that was in the original group cages. The dustbath was filled daily with either sawdust or peat moss, depending on the dust substrate with which they were provided in their previous cages. The experimental facility consisted of three tiers, with five pairs of cages on each tier. Figure 5.2 shows the housing set-up for the experiment. As it was undesirable to have the hens housed in different rooms (because of the possible effect of room) an attempt to eliminate cross contamination of substrates was made by housing the sawdust hens on the top tier, with the remaining hen pair in the cage on one end of the second tier. Although this arrangement could confound results due to a tier-treatment effect, it was
important to ensure the sawdust was not contaminated with peat moss. Peat moss hens were housed on the bottom tier with the remaining hen pair housed in the cages on the other end of the second tier. Spare hens were housed in the centre, middle tier cages. Each hen pair (i.e. the dominant and subordinate hens chosen from the same pre-experimental group-housed cage) was randomly allocated into a pair of adjoining cages. Pairs of cages were separated by metal mesh, which allowed limited tactile contact between neighbours. Each pair of cages was separated from other pairs by solid metal walls. Dominant hens were chosen to be test subjects for the experiment while the subordinate neighbour of each dominant hen was designated to be the ‘social option’ in the Y-maze for the dominant individual during testing. This pairing was determined so as to ensure the ‘social option’ in Y-maze testing was attractive and non-threatening to the test bird, as it has previously been demonstrated that hens will avoid unfamiliar hens or familiar hens that are dominant to them (Dawkins, 1982; Bradshaw, 1992; Grigor et al., 1995). From here onwards, dominant hens will be referred to as test hens and subordinate hens will be referred to as non-test hens. Hens were checked, fed and dustbaths refilled at approximately 1030 h each morning. Egg production for each test hen was recorded daily. The hens were kept in a constant environmental temperature of about 22°C (mean 22.2°C, range 17.6°C to 27.8°C) and a light:dark cycle of 16:8 h, with lights coming on at 0500 h.
<table>
<thead>
<tr>
<th>TOP TIER</th>
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<tr>
<td>Cage No.</td>
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<td>1B</td>
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<td>2B</td>
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<td>3B</td>
<td>4A</td>
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<td>SD</td>
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<td>7A</td>
<td>7B</td>
<td>8A</td>
<td>8B</td>
<td>9A</td>
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<td>PM</td>
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<td>12A</td>
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</tr>
</tbody>
</table>

**Figure 5.2** Set up of experimental hen cages. Each box represents a cage. The sawdust (SD) hens were housed above the peat moss (PM) birds so as to prevent sawdust being contaminated by peat moss. Spare hens were housed in the centre cages. Dashed vertical lines denote mesh dividers (between a dominant test hen and a subordinate neighbour). While solid vertical lines denote solid walls (between pairs of cages).
5.2.2 Pre-experimental handling

To ensure hens were accustomed to being handled, all hens (including non-test and spare birds) were handled as described in Chapters 2, 3 and 4.

5.2.3 Preference testing

*Experimental design*

This experiment was a 2 x 3 factorial design comparing peat moss and sawdust and the interval between tests (every day, alternate days or every third day) (Table 5.1). This experiment was conducted over two periods with each hen allocated to a different interval treatment in each period. Hens were randomly allocated to a treatment in each period. Only birds that had had exposure to peat moss were allocated to peat moss treatments and likewise for sawdust.

**Table 5.1** Description of Y-maze treatments

<table>
<thead>
<tr>
<th>Dust substrate</th>
<th>Interval of testing</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Every day (1)</td>
<td>Every second day (2)</td>
<td>Every third day (3)</td>
</tr>
<tr>
<td>Peat moss (PM)</td>
<td>PM1 (n=2 per period)</td>
<td>PM2 (n=2 per period)</td>
<td>PM3 (n=2 per period)</td>
</tr>
<tr>
<td>Sawdust (SD)</td>
<td>SD1 (n=2 per period)</td>
<td>SD2 (n=2 per period)</td>
<td>SD3 (n=2 per period)</td>
</tr>
</tbody>
</table>

*Y-maze apparatus*

The Y-maze that was used in the experiments described in Chapters 2, 3 and 4 was utilised in this experiment.

*Y-maze familiarisation*

Test hens were familiarised to the Y-maze once per day over four consecutive days, commencing at 1100 h. In a randomly determined order, each hen was placed in
the start box (SB) of the Y-maze with both arm gates open and no resources present. After 10 seconds the SB gate was opened and the hen was allowed to leave. If the hen had not left within 30 seconds she was gently pushed forward (with a flat hand on the tail). The SB gate was closed once she had left the SB. Each hen was given a total of five minutes in the Y-maze once she had left the SB to freely explore the apparatus.

**Y-maze training**

Throughout Y-maze training and testing, hens were socially restricted in their home cage by placing opaque rubber partitions between pairs of cages and dustbaths were removed from hens’ home cages.

Each hen was randomly allocated one Y-maze arm (left or right) in which the dustbath was always placed. The dustbath was identical to the tray the birds previously had in their home cage and was refilled with either peat moss or sawdust (depending on which substrate the individual hen was allocated) between hens after every training/testing trial. The other arm contained the test hen’s neighbour, which was confined in a mesh-fronted box at the end of the Y-maze arm. If the Y-maze arm in which the social option was placed had previously contained the dustbath, the arm was cleaned, by brushing, so as to ensure no peat moss or sawdust remained in the arm.

Hens were randomly allocated to an interval treatment (n=2 per dust/interval treatment per period, refer Table 5.1). All birds were trained individually, in a randomly determined order for five training sessions per bird. Hens were trained on days that corresponded to the interval of testing treatment. This meant that the commencement of training was staggered so as to ensure all treatments could commence testing on the same day (Table 5.2). The training sessions aimed to ensure that each hen had equal contact with each Y-maze arm so that she learnt what each contained. To accomplish this, in each training session, each hen received two consecutive trials in the Y-maze, in which only one Y-maze arm was open at a time. The arm in which the bird was first allowed access was determined randomly for each bird each training day and was subsequently alternated each training session.

Each hen was placed in the SB for 10 seconds before the SB gate was opened. The hen was allowed 30 seconds to move out of the SB, if she had not, she was gently pushed forward into the choice area. The SB gate was closed after the hen had moved
out. The hen was then given another 30 seconds to move into the open arm, if she had not, she was gently pushed towards the open arm. This continued until the hen had entered the arm (i.e. crossed the choice point). The hen remained in the Y-maze for two minutes before either being returned to the SB for the second consecutive training trial or being returned to her home cage. Two minutes in the arm was chosen based on results from the experiment described in Chapter 2.
Table 5.2  Example timeline for training and testing hens on different interval treatments for one period. Recovery (REC) referred to the period between training and testing periods in which hens had \textit{ad libitum} access to dust and social contact in the home cage.

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<td>RECOVERY</td>
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Y-maze testing

The Y-maze testing procedure was similar to that for training, but both Y-maze arm gates were open when the hen was in the SB. When the hen moved out of the SB and voluntarily entered one of the Y-maze arms, the gate of the arm not chosen was closed and the hen remained in the maze for two minutes.

Y-maze testing occurred over a period of 13 days (Table 5.2). This meant that birds on treatment 1 had 13 test trials, birds on treatment 2 had 7 test trials and birds on treatment 3 had 5 test trials. Although this meant that birds on different interval treatments had different numbers of test trials, this format of testing allowed birds to be tested during the same period, so as to minimise any bias that may have occurred over time. At the completion of the 13-day testing period, hens were allowed 15 days ‘recovery’ (Table 5.2) in which hens had ad libitum access to dustbathing substrate and social contact in their home cage.

The arm and resource choice, time to leave the SB (defined as the time from when the SB gate was opened until the hen moved into the choice area) and time to choice (defined as the time from when the hen left the SB to when she entered one of the Y-maze arms) were recorded via direct observations. In addition, if dust was chosen, the nature of the interaction was also recorded (no interaction, foraging behaviour or dustbathing). Foraging was defined as a hen pecking and/or scratching the dust and a dustbathing bout was defined to commence when the hen performed the first vertical wing shake. This was a different bout definition from that described in Chapter 3 as the first vertical wing shake was easier to record from direct observations of the hens compared to when the dustbathing bouts were observed on video footage (as in the experiment described in Chapter 3). No dustbathing bouts were completed in the two-minute access time with the dust substrate.

5.2.4 Statistical Analysis

One hen was excluded from the study as she did not lay from approximately mid-way through the first testing period through to the end of the experiment. This hen was a peat moss hen and was allocated into third day and second day interval treatments for periods 1 and 2 respectively.
For mean time measurements the main effect of interval treatment was examined using a residual maximum likelihood (REML) mixed model analysis of the logarithm of the mean measurement with a fixed effect for each combination of dust (sawdust and peat moss) and period, a fixed effect of interval treatment and a random effect of bird. The effect of interval treatment was examined using an approximate F test (Kenward and Rogers, 1997), after adjusting for other terms in the model. In analyses where the variance of the bird effect was estimated to be negative this was allowed to stand, by analogy to the standard approach in analysis of variance of balanced experiments. The relationship of the number of trials contributing to a mean and the residual variation was examined graphically, but no pattern was apparent.

A similar approach was used for testing whether interval treatment differed with dust treatment (i.e. the presence of a dust by interval interaction), except that the model included an extra dust by interval interaction term.

The effect of interval on the proportion of trials that dust was chosen was analysed as a binomial logistic general linear model with fixed effects for each combination of dust and period, the effect of each bird and the effect of interval treatment. This analysis included an over-dispersion parameter and the binomial total was taken as the number of tests. Predicted values of treatment were calculated on the logistic scale, after adjusting for other terms in the model, with equal weighting to all birds that had some trials in which social was chosen. The effect of the dust by interval interaction was tested by adding this interaction to the model.

The effects of interval treatment and dust by interval interaction on the proportion of dust-chosen trials where dustbathing occurred were tested in a similar way, with the exceptions of dispersion being fixed at 1 due to the estimated dispersion being less than 1, and side of the maze in which dust was located was used as a covariate. It was not possible to estimate interval effects on the logistic scale because all hens had at least one period in which they either dustbathed in every dust-chosen trial or did not dustbathe on any dust-chosen trial.

The situation for the proportion of dust-chosen trials where foraging occurred was similar, except that side of the maze in which dust was located was not an effective covariate. Only one hen did not have at least one period with foraging occurring in all dust-chosen trials, or with no foraging occurring in any dust-chosen trials. This implied that estimates on the treatment effects could not be made reliably on the logistic scale.
5.3 RESULTS

5.3.1 Choice behaviour and time to choice

Overall, hens predominantly chose the dust substrate to social contact (88.3% substrate vs. 11.7% for social, overall) ($\chi^2 = 57.760$, $P < 0.0001$).

The interval of testing treatment had no significant effect on the choice behaviour for dustbathing substrate or social contact ($P = 0.52$). There was a tendency for hens in the ‘everyday’ treatment to take longer to leave the start box compared to the other interval treatments ($P = 0.060$). However, the time to choice did not significantly differ between interval treatments in both overall trials ($P = 0.17$) and when dust ($P = 0.39$) and social contact ($P = 0.21$) were examined separately (Table 5.3).

Overall, there were no significant differences between sawdust and peat moss treatments in terms of choice behaviour and time to choice (Table 5.3).
### Table 5.3  
Effect of testing interval treatment on the time to choice and choice behaviour of laying hens.

<table>
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<th>Log transformed (Back transformed)</th>
<th>P-value</th>
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<tr>
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<td>Daily</td>
<td>Alternate days</td>
</tr>
<tr>
<td>Mean time to leave start box (seconds)</td>
<td>Log₁₀</td>
<td>0.29 (1.9)</td>
</tr>
<tr>
<td>Mean time to choice (seconds)</td>
<td>Log₁₀</td>
<td>0.23 (1.7)</td>
</tr>
<tr>
<td>Mean time to dust choice (seconds)</td>
<td>Log₁₀</td>
<td>0.19 (1.5)</td>
</tr>
<tr>
<td>Mean time to social choice (seconds)</td>
<td>Log₁₀</td>
<td>0.27 (1.9)</td>
</tr>
<tr>
<td>Proportion of trials dust chosen</td>
<td>Logistic</td>
<td>1.52 (0.82)</td>
</tr>
<tr>
<td>Proportion of dust-chosen trials where dustbathing commenced</td>
<td>Logistic</td>
<td>Not possible to estimate on the logistic scale because all hens had at least one period in which they either dustbathed in every dust-chosen trial or did not dustbathe on any dust-chosen trial</td>
</tr>
<tr>
<td>Proportion of dust-chosen trials where foraging occurred</td>
<td>Logistic</td>
<td>Not possible to estimate reliably because there was only one hen that did not have at least one period in which foraging occurred on all dust-chosen trials, or foraging did not occur on any dust-chosen trials</td>
</tr>
</tbody>
</table>

* Back transformed values in the proportion of trials dust chosen are given for those hens that did not select dust in every test trial (which meant excluding three birds which selected dust on all trials). Values in italics are corrected by including a value for 1 (i.e. dust chosen on 100% of trials) for the excluded three birds.

#### 5.3.2 Behaviour in the Y-maze

The foraging and dustbathing behaviour for each interval and dust treatment are shown in Figure 5.3. Although there was statistically no significant differences between the occurrence of dustbathing and foraging behaviours between either interval or dust treatments (refer Table 5.3), this was most likely due to individual
variation. The individual hen variation in dustbathing and foraging behaviour in the Y-maze is presented in Table 5.4.

**Figure 5.3** Proportion of dust-chosen trials in which dustbathing and foraging behaviour occurred for each dust and interval treatment (raw data presented).

**Table 5.4** Individual variation in dustbathing and foraging behaviour in the Y-maze. Data arranged from least to most foraging behaviour.

<table>
<thead>
<tr>
<th>Bird number</th>
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<th>Proportion of dust-chosen Y-maze trials</th>
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<td></td>
<td>Foraging</td>
<td>Dustbathing</td>
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<td>11</td>
<td>Peat moss</td>
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<td>2</td>
<td>Sawdust</td>
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<td>Peat moss</td>
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<td>12</td>
<td>Peat moss</td>
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5.4 DISCUSSION

The present results revealed that the choice behaviour of hens was unaffected by interval and dust treatments. All hens predominantly chose dust to social contact. While the hens tested daily had a tendency to be slower to leave the start box than the other interval treatments, the time to choice was not affected by either interval or dust treatments.

The choice behaviour of hens revealed that the dust substrate provided in the Y-maze was a more attractive resource for all birds, compared to social contact. Interestingly, the type of dust substrate did not affect choice for dust, which is contrary to expectations.

Although sawdust is a less preferred dustbathing substrate compared to peat moss (Petherick and Duncan, 1989; de Jong et al., 2007), it may not be a lower quality foraging substrate. Petherick and Duncan (1989) found that hens performed more foraging on peat moss and sawdust compared to sand and wood shavings. Using an operant conditioning task, de Jong et al. (2007) reported that hens showed no clear preference for a particular material in which to forage (when offered sand, peat moss, wood shavings or wire floor). Furthermore, hens prefer to perform foraging behaviour in materials that are loose and manipulable (Moffat, 2000). Both peat moss and sawdust are loose and manipulable substrates and results from the present experiment found that hens utilised both dust substrates for foraging (Figure 5.3). Therefore, in contrast to their use as a dustbathing substrate, peat moss and sawdust may be equally attractive as a foraging substrate. Table 5.4 shows that two hens performed dustbathing bouts on most or all of their dust-chosen trials, three other hens performed dustbathing behaviour on about half of their dust-chosen trials and the remaining six hens predominantly foraged when they chose dust. Therefore, the majority of hens preferred to forage rather than dustbathe, or perform foraging and dustbathing on about half of their dust-chosen trials when dust was chosen. The use of dust for both foraging and dustbathing most likely led to the high choice for dust in this experiment, regardless of which dust type was presented.

The behaviour of hens with respect to their use of dust in the Y-maze was not significantly different between interval or dust treatments (Table 5.3). However, looking at the behaviour of each individual in the Y-maze (Table 5.4) revealed that all but one peat moss hen performed dustbathing on at least one dust-chosen trial.
Conversely, only two sawdust hens performed dustbathing, while the other four sawdust hens performed foraging behaviour on all dust-chosen trials. Individual variation in dust substrate use in a Y-maze has been observed previously by Petherick et al. (1990b) and in the experiment described in Chapter 2. Petherick et al. (1990b), who used peat moss for the substrate, suggested that peat moss may “switch on” dustbathing behaviour in some hens, while this is not the case for other hens. Results from the experiment described in Chapter 2 showed that individual variation in dust usage was not just in the Y-maze, but was consistent when hen behaviour was observed in the home cage when their dustbath was refilled.

Another reason why the preference for dust was so high compared to social contact could be due to the extent of social contact available in the home cages, as restriction was only visual; hens still had auditory and olfactory contact with the flock. Furthermore, when in the start box and choice area of the Y-maze, hens had (brief) visual contact with the stimulus hen (the social option) in the Y-maze. Perhaps this brief visual contact (perceiving that there was a hen in the vicinity), coupled with the sound and smell of other hens when in the home cage was sufficient social contact for hens, leading to the low preference for social contact.

Speed of movement may be used as an indicator of motivation (e.g. Petherick et al., 1992). Although hens tested daily had a tendency to leave the start box slower compared to those tested on every second or third day, once in the Y-maze the time to choice did not differ between interval or dust treatments. Even though dustbathing behaviour typically occurs every second day (Vestergaard, 1982), the results indicated that all hens were similarly motivated to obtain a resource in the Y-maze.

The results obtained in this study suggest further research is warranted to better understand the test design factors in preference tests of two attractive dustbathing substrates and testing interval. These methodological issues may influence the results obtained from preference tests, thus leading to spurious results that are not reflective of the animal’s preferences. A similar but larger scale experiment in the future, utilising a resource that may be more competitive with dust (e.g. feed), may help in determining whether lack of differences in the present study was due to the relatively low number of experimental animals. Giving hens the choice between two similarly attractive resources may expose subtle differences in motivation or choice due to the interval between tests. For example, hens may select
dust only for dustbathing when it is offered against food, as foraging would presumably be more rewarding with a food resource compared to dust.

The results from this experiment indicate that neither the interval of testing nor the quality of dustbathing substrate presented affected choice behaviour when social contact and dust was offered as rewards. All hens showed a clear preference for dust to social contact, regardless of the type of dust substrate or interval of testing. However further research on these aspects, utilising a resource that may be more competitive with dust (e.g. feed), is necessary to comprehensively examine the effects of interval and quality of the resource on choice behaviour.
CHAPTER 6

MAJOR FINDINGS, CONCLUSIONS AND RECOMMENDATIONS

6.1 MAJOR FINDINGS AND CONCLUSIONS

The preferences of animals may provide us with an indication of what is required to optimise their welfare. To ensure that the results obtained in preference tests are reflective of the animals’ true preferences, a rigorous methodology is required in which to assess them. The aim of this thesis was to examine some of the fundamental factors of Y-maze preference test methodology and their effects on the motivation and consequent choice behaviour of laying hens. The main results are now summarised and discussed.

6.1.1 Methodological factors

*Quantity of resource*

The quantity of resource in a Y-maze preference test may be defined as the amount of time the animal has access with the resource. Results from the experiment described in Chapter 2 indicated that when given a choice in a Y-maze between a tray of peat moss and social contact, the quantity of peat moss resource affected hen motivation (based on time to choice) and choice behaviour. Hens that received 20 minutes of peat moss access were significantly slower to move through the Y-maze to make a choice compared to hens given more (45 minutes) or less (2 minutes) time with the peat moss resource. Although all hens preferred peat moss to social contact, hens that received 20 minutes of peat moss resource also had a tendency to select peat moss less often compared to hens given more or less time with the resource.

The increased time to choice and tendency to choose peat moss less often indicate that hens that received 20 minutes of peat moss access were less motivated to gain access to peat moss compared to hens receiving 2 or 45 minutes of peat moss.
access. This may have been caused by the repeated interruption of dustbathing midway through the bout. This repeated interruption may have caused the hens to experience frustration and aversion to the peat moss resource. Interestingly, hens that received 2 minutes of peat moss access did not show these signs of reduced motivation. Possibly, hens that interruption of dustbathing at the very beginning of the bout did not lead to frustration as hens did not have enough time to become fully engaged in the dustbathing bout.

The experiment described in Chapter 3 found that there was no evidence that hens showed a preference for an uninterrupted dustbathing condition over the condition involving an interruption mid-way through dustbathing bouts. This result contradicts those presented in Chapter 2. There are several explanations for these contradictory results. Firstly, the hens may have been unable to learn the association between the Y-maze arm, visual cue and length of dustbathing bout in the experiment described in Chapter 3. Or secondly, the interruption of a dustbathing bout does not cause frustration to hen.

The quantity of resource may be a particularly important aspect of test design if the resource-directed behaviour takes a certain amount of time to complete (e.g. dustbathing). By interrupting animals prior to the completion of a behavioural bout, the experimenter may inadvertently be causing the animal to become frustrated. Frustration is an aversive state (Amsel, 1992; Wong, 2000) and previous research has shown that hens will avoid aversive stimuli (e.g. Duncan and Wood-Gush, 1972; Rutter and Duncan, 1992). Therefore, the hens receiving the intermediate resource quantity may have shown indicators of reduced motivation to access the peat moss resource because they found it to be an aversive stimulus due to the repeated interruption mid-way through their dustbathing bouts.

**Quality of resource**

Results obtained in the experiment described in Chapter 5 indicated that, when given the choice between a dust substrate and social contact, hens predominantly preferred the substrate. Although half of the hens were presented with peat moss as the substrate and the other half sawdust, hens’ preference was unaffected by the type of dust substrate presented in the Y-maze. The type of dust substrate provided in the Y-maze also had no effect on the incidence of dustbathing behaviour.
Based on evidence from the literature, the dust substrates provided differed in their quality as a dustbathing substrate (as related to their effectiveness to regulate feather lipids) (van Liere and Bokma, 1987). Moreover, previous research has found that hens prefer peat moss to sawdust as a dustbathing substrate (Petherick and Duncan, 1989; de Jong et al., 2007). Peat moss was chosen as a high quality dustbathing substrate, whilst sawdust was offered as a low quality dustbathing substrate. However, the type of dust presented in the Y-maze did not affect hen choice or motivation (as measured by their speed of movement).

Results from this experiment also revealed that there was large variation in how hens used the dust substrate when they selected it, similar to the variation observed in the experiment described in Chapter 2, with some hens consistent “dustbathers” and others consistent “non dustbathers” (see section 6.1.2 below). In contrast to their use as a dustbathing substrate, previous studies have shown that hens do not have a preference for either sawdust or peat moss for use as a foraging substrate (Petherick and Duncan, 1989; de Jong et al., 2007). Therefore, both dust substrates may be equally attractive as foraging substrates. Although there was individual variation in how hens utilised the dust substrate (see section 6.1.2), the majority of hens (9 out of 11) foraged on half or more than half of their dust-chosen trials. The use of dust substrates for both foraging and dustbathing behaviour probably led to the high choice for dust in this experiment, regardless of which dust type was presented.

**Interval between testing**

Results from the experiment described in Chapter 5 also indicated that the choice behaviour of hens tested every day, every second day or every third day for their choice between a dust substrate or social contact did not differ. Although there was a tendency for hens tested daily to move out of the start box slower than those tested on alternate days or every third day, the time to choice did not differ between hens, indicating that they all had a similar level of motivation.

The intervals between testing treatments in the experiment described in Chapter 5 were based on the reported occurrence of dustbathing behaviour (every second day, Vestergaard, 1982), however, the interval of testing did not impact on the hens’ motivation (as measured by time to choice) or choice. This was probably due to
the substrates being utilised as a foraging substrate and not just a dustbathing substrate as the majority of hens performed foraging behaviour on half or more than half of their dust-chosen trials. Therefore, the lack of effect due to the interval between testing was probably due to the dust substrates being utilised for foraging on most dust-chosen trials, compared to dustbathing.

6.1.2 Animal factors

Social effects

The incidence of dustbathing behaviour in the Y-maze in the experiment described in Chapter 2 was correlated with the familiarity score of hens, which was calculated by the amount of time each hen spent near a familiar or unfamiliar hen in a runway test. The negative relationship showed that hens that preferred to be near a familiar hen dustbathed less in the Y-maze. A possible explanation for this could be related to the individual hen’s fear response in the novel (Y-maze) environment. Although efforts were made to thoroughly familiarise hens to the Y-maze, it is possible the removal of the hens from the home cage and placing them in the Y-maze still caused fear. As hens are unlikely to commence a dustbathing bout unless they feel secure (Olsson and Keeling, 2005), hens that did not or rarely dustbathed may have had a greater fear response compared to hens that dustbathed frequently.

Individual variation

Variation in how hens interacted with the dust substrate in the Y-maze was observed in the experiments described in Chapters 2 and 5. Some hens were classified as “dustbathers” as they commenced dustbathing behaviour in all or most of their dust-chosen test trials. Conversely, other hens were classified as “non dustbathers” as they rarely, if ever, commenced a dustbathing bout in their dust-chosen trials. As all hens performed foraging behaviour and then only some hens commenced a dustbathing bout, the “non dustbathers” may also be referred to as “foragers”. Results from the experiment described in Chapter 2 revealed that this variation in dustbathing behaviour was consistent when hens were observed in the home cage, with hens classified as “dustbathers” performing more dustbathing bouts and more real
dustbathing bouts (opposed to sham) compared to “foragers” during an 11-day pre-experimental observation period.

Interestingly, this type of individual variation in substrate use by hens has only been reported once in the literature. Petherick et al. (1990b) reported this variation between birds and concluded that peat moss “switches on” dustbathing in some hens, while not the case in others.

Individual variation has been reported in other species. Recent research from Hemsworth et al. (2009) report that pigs differ in their preference for food and social contact. In a Y-maze test, it was found that some pigs consistently chose food whereas others consistently chose social contact. During the process of domestication, there may have been processes contributing to the formation of this variation. For example, the artificial selection process for the breeding stock of laying strains has been focussed on selecting animals for egg production, egg quality and food conversion efficiency (Appleby et al., 2004e). However, these animals selected for breeding may also have differed in their behaviour (i.e. dustbathers or foragers) and this would also be passed on to the next generation. Genetic drift, which may be defined as “the random fixation or loss of genes in the population gene pool” (Price, 2002), may have led to the creation of ‘types’ of animals.

From a welfare perspective, this variation in hen preference to perform dustbathing or foraging raises the fascinating question as to whether these two ‘types’ of birds have different welfare requirements. The welfare of an animal may be compromised if it is prevented from performing a highly motivated behaviour (Dawkins, 1988). Therefore, if hens are deprived of the opportunity to perform dustbathing behaviour in an appropriate substrate, potentially hens described as “dustbathers” may suffer more as a consequence compared to hens described as “foragers”.

Moreover, feather pecking (which is the “pecking at and pulling out of feathers of conspecifics” (Rodenburg and Koene, 2004)) has been hypothesised to be a result of redirected foraging behaviour when a suitable foraging substrate is not provided (e.g. Blokhuis and Arkes, 1984; Huber-Eicher and Wechsler, 1997; 1998). For example, Huber-Eicher and Wechsler (1997) found that the providing laying hen chicks with a foraging substrate (straw) reduced the incidence of feather pecking compared to when a dustbathing substrate (sand) was provided. If there are two ‘types’ of hens, potentially hens described as foragers may have a higher probability
of developing feather pecking behaviour compared to hens described as dustbathers when they are deprived of a suitable foraging material.

Side bias

A bias, or preference, to the right was observed in the experiment described in Chapter 3. Hens chose the right maze arm significantly more often compared to the left arm. No other experiments described in this thesis found such a bias.

The experiment described in Chapter 3 was the only experiment which presented identical resources (peat-filled trays) in each Y-maze arm, therefore, it is possible that hens could not differentiate between them and thus exhibited the preference to select the right Y-maze arm. Therefore, this preference for the right side may reflect an underlying side preference when offered choices differ little. There are few other reports of side bias of hens in the literature. Petherick et al. (1993b) reported that hens made more correct choices in a Y-maze test (i.e. selected the resource of which they were deprived) more often on the right compared to the left of the Y-maze. Side biases are attributed to lateralization of the brain. The right eye/left brain hemisphere is reported to deal with complex tasks whereas the left eye/right brain hemisphere deals with potential threats (e.g. Rogers, 2004). The task in this experiment required hens to learn and remember which of the two peat-filled dustbaths was associated with which dustbathing bout (interrupted after 2 or 15 minutes or uninterrupted). It was therefore a more complex task compared to the other experiments described in this thesis. Therefore, it is possible that the hens in the Y-maze used their right eye (and left brain hemisphere) to evaluate the maze as a complex/learning task was involved, which might in turn have led to the expression of a bias to the right.
6.2 RECOMMENDATIONS AND FUTURE RESEARCH

6.2.1 Methodological factors

Resource properties

The experiment described in Chapter 2 reported that the quantity of reward in a Y-maze test had an impact on animal motivation. This experiment altered the quantity of dust substrate resource and the reduced motivation is suggested to be a result of the repeated interruption of dustbathing bouts. Therefore, these results suggest that it is likely that the quantity of resource will only affect animal motivation if the resource presented initiates a behavioural bout and if the resource-directed behaviour is initiated and terminated by endogenous factors due to the required amount of time to complete the bout. Interruption prior to the completion of the behavioural bout may influence the animals’ subsequent motivation to access the resource, like that observed in the experiment described in Chapter 2.

This implies that future hen preference studies incorporating a dustbathing substrate should make the quantity of dust substrate resource as an important consideration when designing the methodology. Providing an amount of time with the substrate that may cause interruption of dustbathing mid-way through the bout should be avoided, as this may impact on hen motivation, like that observed in Chapter 2. Interestingly, providing hens with two minutes of peat moss access seemingly did not influence motivation. This may be a more practical amount of time in future preference studies.

Results obtained from the experiment described in Chapter 5 indicated that the quality, or type, of dust substrate presented in the Y-maze did not affect hen motivation or choice. The two types of substrates differed in their quality as a dustbathing substrate, however, the majority of hens used the dust for foraging behaviour. Therefore, the choice for resources in preference tests may be affected by the type of resource presented, however, this may be dependent on how the animal uses/interacts with the resource.

Conducting a similar but larger scale experiment as the one described in Chapter 5, examining the effects of interval between testing and quality of dust
substrate, is also warranted due to the relatively small number of experimental animals used in that experiment (n=11). Perhaps replacing social contact with a more attractive and thus more competitive resource (e.g. food) may aid in observing differences in motivation or choice due to the quality of dust substrate and/or interval of testing if this was a real effect. Food was not presented as a Y-maze resource in any of the experiments described in this thesis, as it was proposed that it would be a highly attractive resource and there would be a very high preference for it. However, results from this thesis indicate that peat moss is also a highly attractive resource, so comparing food and peat moss may result in a more competitive preference test. If there is a real effect of interval between testing and/or quality of resource, it will have important implications for the selection of testing interval in the future or type of resource presented in preference studies.

Side bias

Although only one experiment described in this thesis found a side preference, it is an important factor to take into account in the future, particularly when the resources presented in the test differ little or the task the animal is required to perform is relatively complex (like that in the experiment described in Chapter 3). This is to ensure that animals are selecting Y-maze arms for the resource that they contain, not because of the side. Perhaps examining the animal’s choice in the testing apparatus prior to placing resources in it will provide an indication of an animal’s preference and if a side bias exists. Future preference studies should include the side of choice in their analysis as well as the resource choice to ensure a side bias, if it exists, is identified.

Other methodological factors

All the experiments conducted in this thesis attempted to thoroughly familiarise the hens to both handling and the testing apparatus. This was done to ensure that hens would be minimally fearful of the novel environment. Out of all the experiments described in this thesis, only three hens from one experiment had to be excluded as they failed to move voluntarily in the Y-maze. These hens were sourced from a commercial farm as ‘end of lay’ birds, so it is likely that their prior experience
influenced them. Therefore, the pre-experimental handling and familiarisation seemed to successfully familiarise most hens with being handled and being placed in the Y-maze. However, studies in the past have not conducted such familiarisation techniques (e.g. Signoret, 1970) and it is possible that fear and/or stress from human handling and novelty may have impacted on the preference or behaviour in the test. In order to minimise the effect of animal fear or stress in a novel testing situation, it is recommended that animals should be familiarised prior to the experiment.

Preference tests in the future should also ensure that the task required of the animals in not too complex. Fraser and Matthews (1997) suggested that the task in a preference of motivation study should not lie outside the animals’ cognitive ability. It is possible that the task of learning to associate dustbaths with dustbathing bouts in the experiment described in Chapter 3 was too complex for the hens and thus no preference was observed.

There are also a number of other aspects of the design of Y-maze test methodology that have yet to be examined. One such factor, which may be particularly important, is the social context of preference studies. Animals (and humans) often make decisions as a group (e.g. Conradt and Roper, 2005; Conradt and List, 2009; King and Cowlishaw, 2009), however, most preference studies, including the experiments described in this thesis test animals singly. This is usually conducted as to make results easier to interpret. The social context of the experiments described in this thesis may have influenced the hens. Hens may not have performed dustbathing in the Y-maze due to being isolated (i.e. tested singly) as dustbathing has been found to be socially facilitated (Duncan et al., 1998) and hens are often observed performing dustbathing simultaneously (Vestergaard, 1982). Alternatively, although hens were familiarised to the testing apparatus, some hens may have experienced fear from being isolated in the novel environment and therefore not engaged in dustbathing. Some studies have shown that animals may work more or less when in the company of conspecific compared to when isolated (Pedersen et al., 2002; Sherwin, 2003). Therefore, the social environment in which preference studies are conducted may well impact on animal motivation or choice. If the resource on offer in a preference test elicits or motivates a certain behaviour that is usually performed as a group or is socially facilitated (e.g. feeding and perhaps dustbathing in the hen), then the social context of the test may be especially important. Potentially, an animal may not choose to perform a socially facilitated behaviour in isolation and therefore may
not select the corresponding resource. This may lead to differences in the preferences of animals when tested individually or as a group. This is a clearly an area for future research.

Diurnal effects may affect the motivation and thus choice behaviour of an animal. The experiments described in this thesis were all conducted from late morning to early afternoon, as to ensure hens were post-oviposition and to coincide with presumed peak dustbathing motivation. However, if the testing was conducted at time prior to or after this, the motivation of hens may have been different. For example, dustbathing occurs from around midday to early afternoon (Vestergaard, 1982). Therefore, if hens were tested in late afternoon they may have not displayed as much dustbathing behaviour and also may not have chosen peat moss as often. Research in this area would be valuable as to determine whether animals do change their preference over time (i.e. during the day). If so, there are clearly implications for the design of future preference studies such as giving the time of day when testing occurs greater consideration in the design of preference tests.

6.2.2 Animal factors

Individual variation

It is interesting that the identification of two ‘types’ of hens, which differ in how they use a dust substrate, was made in the experiments described in Chapters 2 and 5, as there were a relatively small number of experimental animals used in these experiments (15 and 11 respectively). Further research is clearly required to determine if these two ‘types’ of hens do exist by examining a larger number of animals.

If these two ‘types’ of animals do exist, the next step would be to determine whether they differ in other aspects of their behaviour and if so, the consequences. Results from the experiment described in Chapter 2 indicate that these two groups differ in their dustbathing behaviour in the home cage. However, there are numerous other questions. For example, do hens classified as “foragers” spend more time feeding compared to those classified as “dustbathers”? If so, do “foragers” have a higher body weight compared to “dustbathers”? Conversely, as dustbathing functions to maintain plumage, do hens classified as “dustbathers” have better plumage compared to “foragers”? Furthermore, as feather pecking has been hypothesised as
being caused by redirected foraging behaviour, do hens classified as “foragers” perform more feather pecking compared to those classified as “dustbathers”? Examining these other aspects of behaviour may aid in understanding the differences between hens classified as “dustbathers” and “foragers”.

From a welfare perspective, it would be valuable to examine how hens differ in dust substrate use and how important a dust substrate is for them as they may also differ in their welfare requirements. For example, how do “dustbathers” and “foragers” respond when deprived of their preferred resource? Do “dustbathers” suffer more as a consequence of deprivation of a dustbathing substrate than “foragers”? Conversely, do “foragers” suffer more as a consequence of deprivation of a foraging substrate than “dustbathers”? This may be assessed, for example, by use of behavioural and perhaps physiological assessment measures of hens in response of thwarting access to a foraging or dustbathing substrate. It would be presumed that hens denied access to a preferred material would show greater indicators of frustration or stress compared to hens denied access to a less preferred material.

If this difference in dust usage is a real effect, it also raises some interesting questions about its genetic and/or experiential basis. For example, what influence does genetics and experience play on the formation of the ‘types’ of hens? If there is a genetic link, potentially hens could be selectively bred, which may be especially important if hens classified as “foragers” do have a higher probability of becoming feather peckers.

The experiment described in Chapter 2 also suggests that there may be a relationship between dustbathing bout incidence, preference to be near a familiar animal and fear (of novelty). To examine this relationship, hens should be tested in a runway to determine their preference to be near a familiar or unfamiliar hen and then observed for their dustbathing behaviour in a novel environment (like that of the Y-maze). Finally, to determine if there is a relationship between these factors and fear, hens should be tested for their fear response, perhaps in an open field test.

Other animal factors

There are numerous other animal factors that have the potential to influence the motivation or choice of an animal in a preference test. For example, the effect of genetics has not received much research in terms of assessing preferences. The hens
used in the experiments described in this thesis were all of similar strain. The hens used in experiments described in Chapters 2, 3 and 5 were Brown Hy-Line hens, while the hens used in the experiment described in Chapter 4 were a Brown Hy-sex Ingham strain. Hens were purposely selected to be of similar strain as to avoid differences that may exist between strains. Different strains of the domestic hen have been found to differ in their behaviour (e.g. white hens have been reported to be more “flighty” compared to brown hens, Murphy, 1977). Possibly, these strains also differ in their preferences for resources or their motivation to access them. If so, the strains may need different environmental requirements to optimise welfare.

6.3 Closing remarks

To ensure that a rigorous methodology is available in which to assess the preferences of animals, further research examining design features of Y-maze preference tests, including the above examples, should be conducted to ensure design features of the tests are not unintentionally influencing animal motivation and thus choice behaviour. Once we have a rigorous methodology by which to assess the preferences of animals, we may then be able to identify important factors to include in the environments of animals to improve their welfare.
REFERENCES


Beeby, R., 2007. Move to ban battery hens, The Canberra Times, Canberra, Australia 16/04/07


Dawkins, M. S., 1983b. Battery hens name their price: Consumer demand theory and the measurement of ethological 'needs'. *Animal Behaviour*, 31, 1195-1205


Duncan, I. J. H., 1993. Welfare is to do with what animals feel. *Journal of Agricultural and Environmental Ethics*, 6 (supplement 2), 8-14


Fraser, D., Phillips, P. A. Thompson, B. K., 1993. Environmental preference testing to assess the well-being of animals - An evolving paradigm. Journal of Agricultural and Environmental Ethics, 6 (special supp 2), 104-114


Hughes, R. N., 1965. Spontaneous alternation and response to stimuli change in the ferret. *Journal of Comparative and Physiological Psychology*, 60, 149-150


Hughes, B. O., Appleby, M. C., 1989. Increase in bone strength of spent laying hens housed in modified cages with perches. *Veterinary Record*, 124, 483-484


Hutson, G. D., 1984. Animal welfare and consumer demand theory: are preference tests a luxury we can't afford? Anim Behav, 32, 1260-1261


Jones, R. B., 1984. Open-field responses of domestic chicks in the presence of a cagemate or a strange chick. IRCS Medical Science, 12, 482-483


Jones, R. B., Merry, B. J., 1988. Individual or paired exposure of domestic chicks to an open field: some behavioural and adrenocortical consequences. Behavioural Processes, 16, 75-86


Kerje, S., Carlborg, O., Jacobsson, L., Schutz, K., Hartmann, C., Jensen, P., Andersson, L., 2003. The twofold difference in adult size between the red junglefowl and White Leghorn chickens is largely explained by a limited number of QTLs. Animal Genetics, 34, 264-274


King, A. J., Cowlishaw, G., 2009. Leaders, followers and group decision-making. Communicative and Integrative Biology, 2, 147-150


LayWel. 2006. Overall strengths and weaknesses of each defined housing system for laying hens, and detailing the overall welfare impact of each housing system. Deliverable 7.1. http://www.laywel.eu


Lindqvist, C. E. S., Schutz, K. E., Jensen, P., 2002. Red jungle fowl have more contrafreeloading than white leghorn layers: effect of food deprivation and consequences for information gain. *Behaviour*, 139, 1195-1209


Mohamed, M. A., Hanson, R. P., 1980. Effect of Social Stress on Newcastle Disease Virus (LaSota) Infection. *Avian diseases*, 24, 908-915


Pickett, H., 2007. Alternatives to the barren battery cage for the housing of laying hens in Europe. Compassion in World Farming


Rae, M. 2008 Battery-hen ban bid. The Mercury, 11/03/2008


Vaisanen, J., Jensen, P., 2004. Responses of young red jungle fowl (Gallus gallus) and white leghorn layers to familiar and unfamiliar social stimuli. Poultry Science, 83, 335-343


Wichman, A., Keeling, L. J., 2008. Hens are motivated to dustbathe in peat irrespective of being reared with or without a suitable dustbathing substrate. *Animal Behaviour*, 75, 1525-1533


APPENDIX I

DETERMINATION OF THE MINIMUM HEIGHT OF THE DUSTBATH LIP IN CHAPTER 4

The dustbath lip used in Chapter 4 was attached to the long side of the dustbath in the Y-maze arm, which was closest to the start box/choice point. This was put in place as to prevent hens having visual contact with the contents of the dustbath prior to choosing a Y-maze arm.

![Diagram of Y-maze and dustbath measurements](image)

**Figure A1.1** Measurements of the Y-maze relevant to the calculation of dustbath lip height in Chapter 4.

The dustbath was placed 85 cm from the choice point in the Y-maze arm and the height of the arm entrance was 35 cm (Figure A1.1). The width of the dustbath was 24 cm. To calculate the angle of vision the hens had when they entered the arm, the angle (A) was determined by use of formula (1) from Figure A1.2, with the value “a” as the height of the Y-maze arm entrance and “b” as the distance from the choice line to dustbath in Y-maze arm plus the width of the dustbath (Figure A1.1). This angle was calculated as 18.11°. The height of the small triangle in Figure A1.1 was then determined by use of formula (2) in Figure A1.2, using “b” as the width of the dustbath only. This final value, 7.20 cm, was the minimum height that the hen could not view past to have visual contact with the contents of the dustbath from the choice line.
Figure A1.2  Trigonometric formulae used in calculation of the dustbath lip height in Chapter 4.

Formulae used:

(1) \( \tan A = \frac{a}{b} \)

(2) \( a = \tan A \times b \)