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scientific assessment of
poultry welfare**

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Improving the scientific assessment of poultry welfare

Project No. 04-18

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Executive Summary

While there are several concepts of animal welfare in the literature, scientists have basically used two methodologies to study animal welfare: the welfare of animals has been assessed on the basis of either biological functioning or animal preferences. The first approach is an integrated one measuring behavioural, physiological, health and fitness responses to assess biological functioning on the basis that difficult or inadequate adaptation will generate welfare problems for animals. The second uses animal preference (and behavioural demand) testing on the basis that animal preferences are influenced by the animal's emotions, which have evolved to motivate behaviour in order to avoid harm and facilitate survival, growth and reproduction.

This scientific uncertainty in relation to animal welfare concepts or views does not necessarily diminish the robustness of the research utilising criteria or methodologies promulgated by these different views or concepts. However, it does raise the question of the relatedness of these concepts. In other words, is biological dysfunction associated with or does it lead to negative affective states and vice versa? Thus, are the resultant methodologies measuring the same state(s) in the animal? Alternatively, if different methodologies measure different aspects of the animal's welfare, which aspects are most important for overall welfare? Research utilising well-accepted stress models is required to understand the relationships between these concepts and methodologies. Fundamental research, with the overall objective of integrating these criteria and developing a broader consensus on animal welfare methodologies, may assist in reducing the interpretative differences in animal welfare science, and this is the general aim of this project.

To examine the relationships between two concepts of animal welfare, the biological functioning and feelings-based concepts, this project aimed to test the hypothesis that deprivation of a preferred resource results in biological dysfunction. A range of behavioural, physiological and fitness responses were used to assess biological functioning in this project, while bird preferences were assessed by offering the birds a choice of resources in a series of Y maze trials. Since preference testing methodologies are less well established than those utilized to assess biological functioning, a significant component of this project was committed to developing a valid and easily applied methodology to assess choice behaviour in poultry. A valid methodology was considered on the basis of (1) the consistency of choice behaviour, both within and between animals, and (2) animals consistently chose the expected desirable resource.

Y-maze tests were used in the first two experiments to examine preferences for feed, social contact and a dustbathing substrate (sawdust). The results of these two experiments indicate that laying hens preferred feed over social contact or dust, irrespective of restriction of any of these resources. Furthermore, the hens were quicker to make feed choices compared with dust or social contact choices. In the social contact and dust comparison, restriction of dust increased choice of dust in the 24 trials, suggesting that dust restriction increased the birds' motivation to access dust. However, since the overall choice for dust and social contact was not significantly different from random choices (or chance), this effect of dust restriction cannot be attributed to a higher preference for dust over social contact, although birds were quicker to make dust choices than social contact choices. The consistency of results across these experiments, together with the results of other preference methodologies in the literature, illustrate the reliability of this methodology for determining the relative choice behaviour of resources for laying hens. However, additional evidence, particularly on the occurrence of abnormal behaviour, stress physiology and health, when restricted of the resource of interest would be useful in providing a more comprehensive assessment of the impact of the restriction on animal welfare.

The quantity of reward in a Y-maze preference test refers to the quantity of time the animal is allowed contact with its chosen resource. This value may potentially affect the choice behaviour of an animal in a preference test by making the resource more or less attractive. The third experiment in this project examined the effects of quantity of reward in a Y-maze preference test on laying hens. The resources in the Y-maze were dust (a tray of peat moss) or social contact (the presence of a familiar subordinate hen). Hens were allocated to one of three treatments, which differed in the quantity of time allowed

with the dust resource when it was chosen; ‘short’ (2 minutes), ‘intermediate’ (20 minutes), ‘long’ (45 minutes). The quantity of reward for social contact remained 5 minutes for all birds. During testing, hens were deprived of both dust and social contact in their home cage. All hens chose dust significantly more than chance level, however, the intermediate treatment had a tendency to choose social contact more often than the other treatments. In addition, the intermediate treatment was slower to move through the Y-maze compared to the other treatments. Overall, these results indicate that while there was no significant effect of the quantity of reward on choice behaviour, birds which received the intermediate reward quantity, when compared to the other treatments, showed evidence of reduced motivation to obtain dust as demonstrated by their tendency to choose social contact more often and their reduced speed of movement. The results of this preliminary study show that the quantity of reward has the potential to affect the choice behaviour of hens for peat moss and social contact. The results from these three experiments also highlight the need to understand the effects of design features of preference tests (e.g. time with resource, social context, resource deprivation level, etc.) on the choice behaviour of laying hens in Y-maze preference tests. This knowledge is necessary if the measurement of the choice behaviour is to be used to answer questions about animal welfare. Further studies in a PhD program at the University of Melbourne are continuing to study the effects of design features of preference tests on the choice behaviour of laying hens in Y-maze preference tests.

The ultimate aim of this project was to study, using well-accepted stress models, the relationships between two concepts of animal welfare, the biological functioning and feelings-based concepts. This was achieved by examining the question of whether deprivation of preferred resources resulted in biological dysfunction. Thus the effects of deprivation of a dust-bath or social contact on biological functioning of birds that either prefer dust-bathing or social contact was studied in the fourth experiment. The preferences of 40 hens for a dustbath or social contact was studied and 24 of these birds were selected: 12 birds that chose social contact in the majority of Y maze trials (labelled “social preferred” group of birds) and 12 birds that chose dust in the majority of Y maze trials (labelled “dust preferred” group of birds). Over a 6 week period, half of each of the two groups of birds (ie. 6 social preferred and 6 dust preferred birds) was housed in one of the two following restrictions, social restriction only or dust restriction only. The effects of these restrictions on these two types of birds on the behaviour and stress physiology of laying hens were studied. The behaviour and physiology data are presently being analysed and will be reported in June 2009 to the Poultry CRC as an addendum to this report.

This fifth and final experiment in this Poultry CRC project will contribute to our understanding of the effects of deprivation of highly preferred resources on biological functioning and thus provide an insight into the relationship between these two methodologies of animal welfare assessment. The finding that deprivation of the resources most preferred by the animals results in biological dysfunction would lead to the development of a broader scientific consensus in that either of these two methodologies can be used independently or together to judge animal welfare in research settings (and in screening tools in the field).

Preference methodologies may provide a reliable measure of animal choices. However, with our present limited knowledge of the relationship between these two methodologies, in order to be confident that the outcomes of preference tests reflect the welfare requirements of the animal, additional evidence, particularly on the occurrence of abnormal behaviour, stress physiology and health when restricted of the resource or the behaviour of interest, would be prudent to provide a more comprehensive assessment of the impact of restricting a resource or behaviour on animal welfare.

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Introduction

Concepts and measurement of animal welfare

There is considerable uncertainty within science on the concept of animal welfare (Fraser, 2003; Sandoe et al., 2004). Scientists differ in their views on how animal welfare should be measured or judged, with three prominent concepts of animal welfare in the literature: the welfare of animals is judged on the basis of (1) how well the animal is performing from a biological functioning perspective; (2) affective states, such as suffering, pain and other feelings or emotions; and (3) the expression of normal or 'natural' behaviours.

The first concept, which is often called the biological functioning concept, is underpinned by the definition of Broom (1986), "The welfare of an individual is its state as regards its attempts to cope with its environment". The 'state as regards its attempts to cope' refers to both (1) how much has to be done in order to cope with the environment and includes responses such as the functioning of body repair systems, immunological defences, physiological stress responses and a variety of behavioural responses and (2) the extent to which coping attempts are succeeding and this includes the lack of biological costs to the animal such as deterioration in growth efficiency, reproduction, health and freedom from injury. Therefore, a key precept in this concept is that animals use a range of behavioural and physiological responses to assist them in coping with environmental conditions, and while biological regulation in response to environmental change is constantly occurring, adaptation is not always possible. When homeostasis fails, there is damage, disease or even death (Broom and Johnson, 1993; Hemsworth and Coleman, 1998; Hemsworth, 2003). This definition of Broom's (1986) is not dissimilar from the one recently endorsed by the 172 member countries of the OIE (the World Organisation for Animal Health, 2008): "Animal welfare means how an animal is coping with the conditions in which it lives. An animal is in a good state of welfare if (as indicated by scientific evidence) it is healthy, comfortable, well nourished, safe, able to express innate behaviour, and if it is not suffering from unpleasant states such as pain, fear, and distress. Good animal welfare requires disease prevention and veterinary treatment, appropriate shelter, management, nutrition, humane handling and humane slaughter/killing. Animal welfare refers to the state of the animal; the treatment that an animal receives is covered by other terms such as animal care, animal husbandry, and humane treatment."

By using the biological functioning approach, the risks to the welfare of an animal imposed by an environmental challenge can be assessed at two levels (1) the magnitude of the behavioural and physiological responses and (2) the biological cost of these responses. These behavioural and physiological responses include the abnormal behaviours, such as stereotypies and redirected behaviours, and the stress response, respectively, while the biological cost includes adverse effects on the animal's ability to grow, reproduce and remain healthy and injury-free.

The second concept, often called the feelings-based concept, defines animal welfare in terms of emotions and emphasizes reductions in negative emotions, such as pain and fear, and increases in positive emotions such as comfort and pleasure (Duncan and Fraser, 1997). Duncan (2004) has argued that animal welfare ultimately concerns animal feelings or emotions as follows. All living organisms have certain needs that have to be satisfied for the organism to survive, grow and reproduce and if these needs are not met, the organism will show symptoms of atrophy, ill-health and stress and may even die. Higher organisms (vertebrates and higher invertebrates) have evolved 'feelings' or subjective affective states to motivate behaviour to meet these needs.

Measuring the preferences of animals, using preference tests and behavioural demand testing (Dawkins 1980; Matthews and Ladewig 1994), has been used by scientists to assess animal welfare predominantly on the basis that these preferences are influenced by the animal's emotions, which have

evolved to motivate behaviour in order to avoid harm and facilitate survival, growth and reproduction. For example, preference testing using a Y-maze apparatus that allows a choice between access to two different resources has been used to provide information about specific features in the animal environment, such as flooring (Hughes and Black, 1973; Hutson, 1981), restraint methods (e.g. Pollard et al., 1994), handling treatments (Rushen, 1986) and ramp design (Phillips et al., 1988), with the overriding objective of optimising the environment for animals. For laying hens it includes light sources (Widowski, et al., 1992), ammonia levels (Kristenson et al., 2000), group size (Dawkins, 1982), perches (Lambe and Scott, 1998) and nest box design (Struelens et al., 2005).

While the consistent choice or preference of one resource over another indicates the animal's relative preference, some have argued that a problem with examining animal preference is that the expression of a preference does not indicate how important the resource is to the animal (e.g. Matthews and Ladewig, 1994). 'Behavioural demand' studies, for example in which operant conditioning techniques such as pecking at a key or pushing through a weighted door have been used to allow the animal to learn to perform a response to gain access to a resource, have been used in an attempt to quantify the animal's level of motivation to access or avoid the situation being tested (Dawkins, 1983; Matthews and Ladewig, 1994; Fisher and Hogan, 2003). Many of these techniques were first developed using laying hens (Dawkins, 1983; Duncan and Kite, 1987; Lagadic and Faure, 1987; also see review by Cooper and Albentosa, 2003).

While not well enunciated, the third concept promotes the principle that animals should be allowed to express their normal behaviour. In the early literature, the view that animals should perform their full 'repertoire' of behaviour was very common, however there is broad agreement within science that it is often difficult to attribute actual suffering when the expression of certain behaviours is prevented or is absent when it would be expected to be present (Dawkins, 2003). Furthermore, as discussed by Dawkins (1980), 'wild' behaviour may represent an animal's efforts to survive in a life and death struggle or contest and therefore some 'natural' responses are adaptations to cope with extreme adverse situations. More recently the emphasis has been on behavioural indicators of poor coping such as fearfulness, aggression and stereotypies (EFSA, 2005).

Related to this notion of the importance of displaying normal behaviour is that of 'behavioural (or ethological) need'. The term 'behavioural need' arose in response to the Brambell Committee report where it was proposed that animals have "natural, instinctive urges and behaviour patterns" and that animals should not be kept in conditions that suppress these behaviour patterns (Brambell et al., 1965). From its inception, the term was highly debated and often criticized for its lack of both clear definition and scientific foundation (Dawkins, 1983). Over time, there was some consensus that the term 'behavioural need' should refer to specific behaviour patterns that may be important for animals to perform and that, when prevented, would result in frustration or some negative psychological state that would cause suffering and impair welfare (Dawkins, 1983; Hughes and Duncan, 1988; Jensen and Toates, 1993). Dawkins (1990) and Fraser and Duncan (1998) suggested that behavioural 'need situations', that is behaviour associated with intense negative emotions, likely evolved for those behaviours where immediate action is necessary to cope with a threat to survival (e.g. escape from a predator) or reproductive fitness (e.g. nesting) while other types of behaviour that can be performed when opportunity arises (e.g. play, grooming) are more likely to be associated with positive emotional states. More recently the term 'behavioural need' has been used to refer to "instinctive behaviours that are performed even in the absence of an optimum environment or resource" (Weeks and Nicol, 2006; also LayWel 2006) and behavioural 'priorities' to refer to behaviour, or resources that accommodate the behaviour (for example a nest box or litter), that animals have been shown to be willing to work for in demand studies (Cooper and Albentosa, 2003; Weeks and Nicol, 2006). By these definitions, any consequences of depriving the animal of performing the behaviours are not considered, which is an important distinction from earlier concepts of behavioural needs. For the laying hen, Weeks and Nicol (2006) suggested that while nesting is a behavioural 'priority', dust bathing, perching and foraging are behavioural 'needs'. Thus, while the consequences of depriving animals of the opportunity to perform these behaviours has not been addressed, there still appears to be a lack of consensus on how to define and provide scientific evidence for behavioural 'needs'.

The so-called 'five freedoms', which were derived from the Brambell Committee report (Brambell et al., 1965) to the UK Parliament, were proposed by the UK Farm Animal Welfare Council in 1993 (FAWC, 1993) to protect the welfare of animals. These freedoms, that is, freedom from hunger and thirst, from discomfort, from pain, injury and disease, to express normal behaviour, and from fear and distress, encompass the principle of a lack of suffering and have intuitive appeal. The five freedoms include aspects of the three concepts of animal welfare, described above. However, it is unfortunate, in terms of a consensus on animal welfare assessment, that there has been little attempt to define the levels of freedom that are desirable together with the adverse consequences of not providing such freedoms.

Development of welfare methodologies

While there are several concepts of animal welfare in the literature, scientists have basically used two methodologies to study animal welfare: the welfare of animals has been assessed on the basis of either biological functioning or animal preferences. As discussed earlier, the first approach is an integrated one measuring behavioural, physiological and health and fitness responses to assess biological functioning on the basis that difficult or inadequate adaptation will generate welfare problems for animals. The second uses animal preference (and behavioural demand) testing on the basis that animal preferences are influenced by the animal's emotions, which have evolved to motivate behaviour in order to avoid harm and facilitate survival, growth and reproduction. These two methodologies and their development will be discussed in more detail here.

Biological functioning studies

The rationale for this approach is the animal welfare definition of Broom (1986), "The welfare of an individual is its state as regards its attempts to cope with its environment". The 'state as regards attempts to cope' refers to both (1) how much has to be done in order to cope with the environment and includes responses such as the functioning of body repair systems, immunological defences, physiological stress responses and a variety of behavioural responses and (2) the extent to which coping attempts are succeeding and this includes the lack of biological costs to the animal such as deterioration in growth efficiency, reproduction, health and freedom from injury. Thus using this functioning approach, which is obviously based on the functioning-based concept of animal welfare, the risks to the welfare of an animal imposed by an environmental challenge can be assessed at two levels (1) the magnitude of the behavioural and physiological responses and (2) the biological cost of these responses. These behavioural and physiological responses include the stress response while the biological cost includes adverse effects on the animal's ability to grow, reproduce and remain healthy and injury-free.

Research on two factors that are likely to affect the welfare of commercial pigs, fear and space, will be briefly considered here to demonstrate this approach in assessing welfare risks in animals. Handling studies on pigs generally indicate that negative handling treatments which resulted in avoidance of humans, also produced either a sustained elevation in the basal free cortisol concentrations or enlargement of the adrenal glands, together with depressions in growth and reproductive performance. These results indicate that the mechanism responsible for the adverse effects of high fear on the productivity of pigs appears to be a chronic stress response. The results also show that negative handling is a potent stressor for pigs with marked effects on behaviour (fear or avoidance responses to humans) and stress physiology (chronic stress responses) which in turn can limit a number of fitness variables such as growth and reproduction. Similar results have been seen in other farm animal species (see Hemsworth and Coleman, 1998).

There is evidence of a chronic stress response, based on prolonged elevation in plasma cortisol concentration and reduced reproductive performance in gilts if space allowance is insufficient (1 m²/pig, Hemsworth *et al.* 1986; and less than 1 m²/pig, Barnett *et al.* 1992). Taylor *et al.* (1997) found that reducing space allowance for groups of 10 sows from 2.0 to 1.2 m²/pig increased aggression. Similarly, Weng *et al.* (1998) reported increased aggression and injuries in sows with decreasing space allowance. These studies show that space allowance is an important factor affecting both the behavioural and physiological responses of pigs and consequently their biological fitness.

These studies on pig handling and housing demonstrate the potential value of this approach of studying both the behavioural and physiological responses and the fitness effects to assess the animal welfare implications. In other words, these studies illustrate this functional approach in which the “state” of an animal as regards “its attempts to cope with its environment” are measured as in the definition of animal welfare proposed by Broom (1986). It is difficult to argue that if such practices affect the fitness of the animal through a range of long-lasting behavioural and neuroendocrine responses that the welfare of the animal is not seriously at risk.

Many would agree that since this approach emphasizes difficulties caused by long-term stimulation, such as housing and regular handling practices, it is likely to identify the most serious situations in which welfare is at risk. In contrast, some may argue that less severe or less prolonged challenges may not be detected with this approach. If this is the case, it could be logically argued that any risks to welfare are relatively low, within the constraints of current methodologies. The single imposition of say a husbandry procedure, because it generally involves short-term stimulation, is obviously less serious to animal welfare since recovery normally occurs, albeit perhaps with a biological cost. However, for short term animal welfare issues involving acute stress, such as painful husbandry procedures, there is considerable agreement on the need to assess animal welfare from a perspective of biological functioning (Mellor *et al.*, 2000). For example, in situations where a husbandry procedure such as mulesing of sheep and dehorning of cattle (Chapman *et al.*, 1994; Goonewardene and Hand, 1991; Paull *et al.*, 2007; Sylvester *et al.*, 1998; Hemsworth *et al.*, 2008) results in substantial and sustained biological responses with consequent adverse effects on fitness (eg growth and gait particularly with mulesing), while the animal has been able to cope, the effects on behaviour, physiology and fitness indicate the magnitude of the challenge to the animal. This challenge reflects the welfare consequences and thus such information can be used to identify alternative techniques or pain relieving procedures to reduce the adverse welfare effects of the practice on the animal.

This approach to welfare assessment has been criticised on the basis that it does not adequately include feelings. However, emotions are part of the body’s regulatory system and together with a range of learning processes function to assist animals in avoiding potentially harmful situations or recognising potentially beneficial situations (Cabanac, 1979). Emotions such as fear and pain clearly have marked effects on the biological responses and fitness of the animal. The effects of handling farm animals as discussed previously clearly indicate the profound effects of fear on stress physiology and fitness. A consistent finding in biological psychiatry is that the physiology of the HPA axis is altered in humans with major depression (see Parker *et al.*, 2003). These findings included a sustained elevation of basal cortisol concentrations and enhanced adrenal responsiveness to ACTH. While depression in humans can be difficult to define, emotions that characterise the condition include sadness and despair, the opposite of joy and contentment (Myers, 1998). Thus, an animal’s attempts to cope with difficulties include an integration of feelings into its behavioural and physiological responses.

Therefore, using this approach, the risks to the welfare of an animal imposed by an environmental challenge can be assessed at two levels (1) the magnitude of the behavioural and physiological responses and (2) the biological cost of these responses. These behavioural and physiological responses include the stress response while the biological cost includes adverse effects on the animal's ability to grow, reproduce and remain healthy and injury-free.

Preference studies

In contrast to the methodology utilized to assess biological functioning, the preference testing methodologies are less well established. Over the last 30 years there has been growing interest in the measurement of the preferences of farm, laboratory and captive animals to answer questions about animal welfare. The rationale for using animal preference tests to study animal welfare is basically three-fold. Some scientists argue that animal preferences can be utilised on the basis that these preferences are influenced by the animal’s emotions (or feelings), which are prime determinants of its welfare (Duncan and Petherick, 1991). Thus proponents of the animal feelings concept, which was discussed earlier, advocate that since animal preferences are influenced by animal emotions and that preference tests can indirectly measure emotions, understanding animal preferences allows inferences to be drawn on animal welfare. While not contrary, others propose that preferences are likely to reflect

important biological requirements of the animal and thus optimise fitness (Broom and Johnson, 1993). Motivation encompasses the internal decision-making process by which the animal chooses to perform a particular behaviour (Barnard, 2004). These behavioural decisions will fluctuate over time, due to a complex interplay of internal and external factors, but the aim of these decisions is to basically maintain homeostasis and optimise fitness and/or positive emotional states. Thus by investigating preferences there may be an opportunity to identify the resources or behaviours that are important to animals. In fact these two views on the value of preference testing are similar in that Duncan (2004) has argued that higher organisms have evolved feelings to motivate behaviour in order for the organism to survive, grow and reproduce. Finally, there is the view that denying an animal access to a resource is likely to adversely affect its welfare more if its preference for the resource is strong rather than weak (Fraser and Matthews, 1997).

The first reports of animal preference testing appeared in the literature in the 1970s (eg. Hughes and Black, 1973; Hughes, 1976; Dawkins, 1976) and many preferences studies have been conducted since, particularly on farm animals. These initial studies stimulated considerable scientific debate relating to conceptual and methodological difficulties (Duncan 1977; Dawkins 1977; van Roojen 1982) and Fraser and Matthews (1997) concluded that the usefulness of preference tests to answer questions about animal welfare is limited by three main issues. First, preference tests should adequately reflect the animal's preference, second, they need to establish how strongly an animal prefers a chosen option, avoids an unpreferred one or is motivated to perform a behaviour, and third, preferences may not correspond to welfare if the choices fall outside the animal's sensory, cognitive and affective capacity or if the animal is required to choose between short- and long-term benefits. These and other issues will be considered later.

There are several methodologies used to study preferences, with the simplest preference study allowing the animal to make a choice between two situations in which the resource is varied. For example, Hughes (1975) found that laying hens preferred a spacious cage to a confined cage and that neither time of day nor strain of bird was influential in this choice. Choice experiments have been used to measure both the choice for and the avoidance of options or environments. A Y-maze apparatus that allows a choice between access to two options has often been used to provide information about specific features in the environment such as flooring on raceways (Hutson, 1981), restraint methods (e.g. Pollard et al., 1994), handling treatments (Rushen, 1986) and ramp design (Phillips et al., 1988). Furthermore, aversion learning techniques have been used to study the animal's motivation to avoid husbandry and handling treatments. For example, Rushen (1986) studied the avoidance of sheep to electro-immobilisation, a procedure in which a pulsed, low-voltage current can be used to immobilise the animal. Sheep were trained to associate a location with a specific treatment and avoidance was assessed based on the effort required to move them repeatedly to the treatment location. Rushen (1986) found over repeated trials that sheep showed increasing avoidance of a location in which they were restrained with electro-immobilisation than to a location in which they were restrained without it.

While the consistent choice or preference of one resource over another or others indicates the animal's relative preference, some have argued that in addition to establishing what an animal prefers, it is important to understand the strength of the preference (eg. Matthews and Ladewig, 1994). To address the question of the strength of an animal's preference, experiments have incorporated varying levels of cost (eg. work effort, time and relinquishing a desirable resource) associated with gaining access to a resource or avoiding aversive stimulation. Sherwin and Nicol (1996) studied the frequency and duration of visits that mice made to various resources, such as food, a conspecific and increased space, when the cost of obtaining those resources varied. In this case, the mice were forced to swim to access the resources, and the distance they had to swim was increased or decreased to alter the cost of the resource to the animal. Bubier (1996) used a multi-choice experiment in which access to the resources, such as food and water, a perch and visual contact with familiar hens, involved either a cost (hens had to squeeze through two dowels) or no cost, to study the strength of the preferences of laying hens.

Dawkins (and others, eg. Matthews and Ladewig, 1994; Cooper and Albentosa, 2003) suggested that food could serve as a standard against which other items can be compared since it is generally agreed that animals suffer if kept without food and that food has an almost inelastic demand. In two experiments on hens, Dawkins (1983) varied the price paid for access to litter by increasing the

duration of feed withdrawal before the test. She found that although hens preferred litter to wire floors, their preference was not strong enough to outweigh the attraction of food and concluded that in both experiments there was no evidence that hens regarded litter as a necessity. Others have shown similar effects (see Cooper and Albentosa, 2003).

In advocating the requirement for quantitative measures of the importance of resources for animals, Dawkins (1983) suggested that these can be derived from measures of demand elasticity. This approach, which is based on consumer demand theory, considers that the demand for a resource is 'inelastic' if it is still chosen (purchased) as the effort (price) increases; in this circumstance the resource is considered as a necessity for the animal. A resource that is chosen less as the effort is increased is described as having an 'elastic' demand curve (eg. luxury items).

This approach of measuring behavioural demand for a particular environment or resource has been extended by studying operant behaviours, such as pecking at or pushing through a weighted door, to quantify the level of demand for the reward. Matthews and Ladewig (1994) studied the behavioural demand functions of pigs for the resources/commodities of food, social contact and a stimulus change (door opening). The amount of work, in the form of pushing a plate, required for access to each reinforcer (resource) was systematically varied. Demand curves, analogous to those used in economics, were derived for each resource by graphing the $\log_{[\text{pig operant responses}]}$ against the $\log_{[\text{fixed ratio operant schedule}]}$. It was found that the demand curve for food was very shallow or flat indicating an inelastic demand for food. In contrast, the demand curve was very steep (ie. a very elastic demand) for opening the pen door and the demand curve for social contact was intermediate. While there is some discussion about which characteristics of the demand curves are more valid (Sherwin, 2004), it is often considered that resources that are more important to animals have slopes closer to zero (ie. shallower).

As mentioned earlier, there are both methodological and conceptual criticisms of preference tests that have to be addressed if preference tests are used to answer questions about animal welfare (Fraser and Matthews, 1997; Dawkins, 2003). Some of these issues and their possible resolution will be briefly reviewed here. Preference tests measure an animal's choice behaviour at a point in time and such measurements run the risk of failing to account for interactions of different motivational states which may influence the behaviour of the animal over time (Hutson, 1984). Furthermore, this short term choice may reflect the animal's proximate (immediate or present) requirements, rather than the animal's ultimate requirements or those necessary for survival, growth and reproduction (Lawrence and Illius, 1997). For example, a hen continues to return to a 'trap-nest' to lay eggs even though it is assumed that the trap-nest is aversive because the hen cannot escape, there is no food or water and it must be handled to be released. The hen's behaviour on entering the nest simply reflects its motivation at the time and not the future consequences (Duncan, 1978). Preference tests need to be comprehensive enough to identify these sources of variation (Fraser and Matthews, 1997).

Preferences may vary with familiarity. The studies by Phillips et al. (1991, 1996) showed that previous experience strongly influenced the sows' initial choice of farrowing crates in which design (solid or open sided crates) and flooring (concrete, plastic coated metal mesh, or bare mesh floors) varied. Similarly laying hens with experience of cages initially preferred this environment to a free range run (Dawkins, 1977). In both cases, the preferences waned as the animals gained experience with the alternatives. Prior experience can be controlled in preference tests by using naïve animals, familiarizing the animals with the resources prior to testing or, as Fraser and Matthews (1997) suggest, prolonged testing.

Preferences for specific resources may also be affected by the context in which the animals are tested. Pedersen et al. (2002) and Sherwin (2003) used operant conditioning techniques to measure the motivation of pigs for food and straw and mice for a running wheel, respectively. These scientists found that the social context at the time of testing affected motivation to access these resources. There is also evidence that the stimuli from the test resources at the time of testing may affect motivation for the resources under study. The motivation of mink to gain access to resources is affected by whether or not they can see the resources (Warburton and Mason, 2003). The context in

which the animals are studied should relate to the commercial conditions in which the experimental question is directed. Indeed, Dawkins (2003) has proposed the development of in situ methods of assessment to allow for the effects of factors, such as the animal's development history and the precise commercial conditions experienced by the animal.

Concerns have been raised about the operant responses used to quantify the level of demand in behavioural demand studies. Fraser and Matthews (1997) suggest that certain operant responses required to be performed to obtain the reward may not be appropriate for certain types of reward. Using the example of hens required to peck a key in order to activate a motorised barrier and enlarge their cage, they questioned whether an alternative response, such as walking, would provide a better measure of the hen's motivation for additional space. Fraser and Matthews (1997) recommend that experiments should be repeated using different methods when there are questions about the appropriateness of the required operant response.

Fraser and Matthews (1997) recognise that limitations in using preference tests arise when animals are exposed to potential dangers or benefits that are beyond their sensory or affective capacity or if the choice requires a level or type of cognitive ability that the animal does not possess. They suggest that the best safeguard is to base preference tests on the types of choices that the species arguably evolved the capacity to make and that the individual animals are accustomed to making.

The conceptual basis of the link between animal preferences and welfare has been criticised because animals may not necessarily prefer or be motivated to obtain resources that are truly in their best interests (Duncan, 1978). For an animal of a wild genotype living in the wild (ie. successfully evolved in the environment), natural selection and behavioural development are expected to produce a set of environmental preferences that promote the health and survival of the individual and offspring. But without such natural selection, should this be expected in domestic animals? While farm animals may have detoxification mechanisms that may effectively deal with some ingested poisons, farm animals can learn to avoid foods that are associated with gastrointestinal distress (Provenza, 1995). There are examples in agriculture where animals, if given appropriate 'choices', are capable of selecting a mixed diet that can ameliorate health issues, such as lameness in broiler chickens (Danbury et al., 2000), that provides physical characteristics to optimise organ development such as gizzard growth in chickens (Cummings, 1994; Wu and Ravindran, 2004) or that allow the optimization of energy and protein requirements for growth and health (Rutter et al., 2004). However, there are examples to the contrary. Using operant conditioning techniques to study the demand of pigs for varying concentrations of sweet solutions, Kennedy and Baldwin (1972) found that a number of pigs had repeated bouts of illness after drinking large amounts of sugar solution. Confined sows show minimal demand for exercise (Ladewig and Matthews, 1996), yet a degree of exercise has been shown to have a longer term beneficial effect on leg strength (Marchant and Broom, 1994).

In concluding on animal preferences, as with biological dysfunction, clarifying the conceptual link between animal preferences and animal welfare is an issue for many. The individual's concept of animal welfare clearly underscores the methodology used to judge or measure animal welfare.

Conceptual uncertainty

These different concepts or views on animal welfare can lead scientists to use different criteria or methodology in assessing an animal's welfare. For short term animal welfare issues involving acute stress, such as painful husbandry procedures, there is considerable agreement on the need to assess animal welfare from a perspective of biological functioning (Mellor et al., 2000). However, for longer term issues disagreement over these welfare concepts, especially when consequent interpretations conflict, can lead to debates concerning animal welfare and the varying interpretations.

This scientific uncertainty in relation to animal welfare concepts or views does not necessarily diminish the robustness of the research utilising criteria or methodologies promulgated by these

different views or concepts. However this conceptual uncertainty has several implications for identifying and resolving genuine risks to an animal's welfare.

First, while there are several concepts of animal welfare in the literature, scientists have basically used two methodologies to study animal welfare: the welfare of animals has been assessed on the basis of either biological functioning or animal preferences. The first approach is an integrated one measuring behavioural, physiological and health and fitness responses to assess biological functioning on the basis that difficult or inadequate adaptation will generate welfare problems for animals. The second uses animal preference (and behavioural demand) testing on the basis that animal preferences are influenced by the animal's emotions, which have evolved to motivate behaviour in order to avoid harm and facilitate survival, growth and reproduction. Therefore, differences in concepts and thus definitions of animal welfare within science lead to differences in the methodology used by scientists to assess animal welfare under different husbandry or housing practices.

Second, differences between policy makers on the concept and definition of animal welfare can lead to disagreement on animal welfare-related policy and legislation. While decisions on specific animal use are affected by a number of considerations including scientific information of the harms and benefits to the animal, differences in concepts, definitions and, in turn, assessment lead to differences between policy makers in industry, community groups and Government in their interpretation of the validity of scientific information arising from a specific methodology. Consequently, these differences between policy makers in interpreting similar information can lead to disagreement on setting or accepting specific animal welfare standards.

These conceptual differences at both scientific and policy levels are illustrated in developments in housing systems for poultry. A recent comprehensive review by European scientists of the literature (LayWel, 2006) provided recommendations on the welfare implications of housing systems for laying hens using the five freedoms as a baseline for animal welfare assessment. The review considered 39 welfare risks under four main categories: 1) Injury, disease and pain (with particular reference to overall mortality, mortality due to feather-pecking/cannibalism, mortality due to disease, infectious disease and use of therapeutic drugs, predation, internal parasites, external parasites osteoporosis/low bone strength, keel bone deformation, bone breaks during lay, bone breaks at depopulation, bumble foot and beak trimming); 2) Hunger, thirst and productivity (with particular reference to feed intake, water intake, FCR and egg production); 3) Behaviour (with particular reference to nest box eggs at peak lay, hens on a perch at night, use of a dust bath, foraging, social, behavioural restriction and injurious pecking); and 4) Fear, stress and discomfort (with particular reference to fearfulness, plasma corticosterone concentration, heterophil to lymphocyte ratio, crowding/suffocation, feather pecking, feather loss, plumage soiling, bumble foot, thermal discomfort, dust, ammonia and dirty eggs). Risks were tabulated separately for conventional cages, furnished cages, non-cage systems and systems with an outdoor run. The report concluded that while all alternative systems have the potential to provide satisfactory welfare for laying hens, conventional cages cannot meet the welfare requirements of hens. However, from the documentation presented in the report, it can be argued that conventional cages perform better in 18 of the 39 risk areas, including those involving mortality, while non-cage and outdoor systems perform better in 9 and 10 categories, respectively, than conventional cages. The conventional cages perform worse for 6 of 7 categories of behaviour, but no evidence is presented that behaviour is more important to welfare than for example, mortality. Using similar interpretations, the European Union Council Directive 99/74/EC proposed that the use of conventional (unenriched) cages will be banned in the European Union by January 2012. In reviewing the development of hen welfare standards in the EU, Savory (2004) concluded that the freedom to 'perform normal behaviour' is often given more weight in interpreting welfare risks than the other four freedoms.

Third, it is important in any welfare monitoring scheme in the field that the emphasis should be on the animal itself and thus on those measures that best reflect a lack of animal suffering. The welfare measures or 'tools' that science develops to evaluate the welfare implications of husbandry and housing practices will obviously be incorporated into welfare assessment and screening tools in the field. Credible field measures are critical in providing assurance to the industry, markets and regulatory authorities. Thus any uncertainty about the validity of the scientific measures on which the

field measures are based will affect the general public, consumer, industry, animal welfare organisations and Government confidence in compliance with specific welfare standards.

Thus, the basis of the methodology used by scientists to assess animal welfare should routinely be provided so that individuals using science in their decision-making appreciate both the rationale for the methodology and its limitations (Fraser, 2003; Sandoe et al., 2004).

Conclusions on welfare assessment

This scientific uncertainty in relation to animal welfare concepts or views does not necessarily diminish the robustness of the research utilising criteria or methodologies promulgated by these different views or concepts. However, it does raise the question of the relatedness of these concepts. In other words, is biological dysfunction associated with or does it lead to negative affective states and vice versa? Thus, are the resultant methodologies measuring the same state(s) in the animal? Research utilising well-accepted stress models is required to understand the relationships between these concepts and methodologies and this is the main aim of this project. Such a consensus within animal welfare science is necessary in using science to assist in establishing welfare standards for animals.

In any consideration of animal welfare assessment, it is useful to reflect on society's objectives in relation to animal welfare. There is a long tradition of ethical thinking in relation to animal use and this has brought us to the present commonly held utilitarian view in many societies that animal management or use by humans is acceptable provided that such management or use is humane (Mellor and Littin, 2004). Thus the priority for many, which is unlikely to diminish in the future, is the avoidance of animal suffering. Notions, such as 'suffering', are often used without being clearly defined. The following two definitions appear to have broad support both in science and the general community. Morton (1998) has defined suffering in terms of 'prolonged adverse physiological and mental states in an animal' and Dawkins (1990) proposes that 'suffering occurs when unpleasant feelings are acute or continue for a long time because the animal is unable to carry out the actions that would normally reduce risks to life and reproduction in those circumstances'. This use of the term suggests that suffering is likely to be synonymous with impaired animal welfare. It is also important to recognise that there is an emerging shift in community values towards not merely minimising suffering in domesticated animals, but also enhancing pleasure in these animals (Tannenbaum, 2001). For many a consideration of animal welfare includes not only the avoidance of suffering, but also the presence of positive subjective emotional states (Duncan, 2004).

While society continues to struggle to identify and agree on minimum welfare standards for its domestic animals, the difficulty of agreeing on desirable animal welfare standards is clearly substantially greater. Nevertheless, the priority for the community is the avoidance of animal suffering. This mandate to avoid suffering is clearly evident in the prevention of cruelty legislation in many Western countries which specifically refers to cruelty in terms of "unreasonable pain or suffering" (e.g. Victoria, Australia (Anonymous, 2007)) or "unnecessary suffering" (the United Kingdom (Anonymous, 1911)). It should be recognised though that the legislation in many of these countries refers to its purpose as not only "to prevent cruelty to animals" but also "to encourage the considerate treatment of animals" (Anonymous, 2007).

In an ethical analysis of animal use, science can provide the factual basis for understanding the impact of a husbandry or housing practice on the animal, particularly its impact on the welfare of the animal. However, there is considerable uncertainty within science on the concept of animal welfare, and these different concepts can lead scientists to use different criteria or methodology in assessing an animal's welfare. To assist in integrating these criteria and developing a broader consensus on animal welfare methodologies, research utilising well-accepted stress models is required to understand the relationships between these concepts and methodologies. In the meantime, the approaches or methodologies that arise from the functioning-based and feeling-based concepts should guide current welfare research methodology. Indeed, while the general public may appreciate that long-term behavioural responses such as stereotypies and chronic stress are 'harmful', there is merit in

understanding what animals prefer since as Fisher and Hogan (2003) note, “we all know which part (approach) will be the most powerful argument for the audience (general public)”. Furthermore, the basis of the methodology used by scientists to assess animal welfare should routinely be provided so that individuals using science in their decision-making appreciate both the rationale for the methodology and its limitations (Fraser, 2003; Sandoe et al., 2004).

Scope of this project

It is clear that research utilising well-accepted stress models is required to understand the relationships between the concepts of animal welfare and their methodologies and this is the main aim of this project. Such a consensus within animal welfare science is necessary in using science to assist in establishing welfare standards for animals. To examine the relationships between two concepts of animal welfare, the biological functioning and feelings-based concepts, this project aimed to test the hypothesis that deprivation of a preferred resource results in biological dysfunction. A range of behavioural, physiological and fitness responses were used to assess biological functioning, while bird preference was assessed by offering animals a choice of resources in a series of Y maze trials.

Since preference testing methodologies are less well established than those utilized to assess biological functioning, a significant component of this project was initially committed to developing a valid and easily applied methodology to assess choice behaviour in poultry. A valid methodology is considered on the basis of (1) the consistency of choice behaviour, both within and between animals, and (2) animals consistently chose the expected desirable resource.

Objectives

The specific objectives of this project were (1) to develop a suitable methodology for preference testing to measure the animal's longer term choice for specific resources and (2) compare the two main approaches to assess welfare: "normal biological functioning" and "preference testing". The finding that deprivation of the resources most preferred by the animals results in biological dysfunction (that is, on-going negative affective states leads to biological dysfunction and vice versa) would lead to the development of a broader scientific consensus in that either of these two methodologies can be used independently or together to judge animal welfare in research settings (and in screening tools in the field).

Outline of the report

This project involved a series of five major experiments. Some parts of these experiments may have appeared in previous progress reports as separate experiments, but for the purposes of this report they have been consolidated and reported here under five experiments.

Methodology

The methods used in this project are described in the methodology section of each experiment.

Experiment 1: The effects of feed and dustbath substrate deprivation on the choice behaviour of laying hens.

1.1 Introduction

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. Therefore, animal preferences will be influenced by the motivational state that may be altered by measures such as resource deprivation (E.g. Petherick *et al.*, 1993).

One method that may assist in determining what is important to an animal, and thus its welfare, is animal preference testing. Measuring the preferences of animals, using preferences and related methodologies such as preference tests and operant conditioning, has been used to study an animal's perceptions of its environment or the adequacy of its environment (Dawkins, 1990; Ladewig and Matthews, 1996). The use of preference methodologies has been justified on the basis that these preferences are either influenced by the animal's emotions, which have evolved to motivate behaviour in order to avoid harm and facilitate survival, growth and reproduction, or reflect important biological requirements of the animal.

Although the use of preference tests has been justified on these grounds, there is still much work to be conducted on the methodological development and improvement of this technique. Rather than comparing the preference or motivation for two resources simultaneously, (i.e. a comparative preference test), this study simplified the procedure and observed hens' choices for a resource when no other resource was present. The two resources under investigation were dustbathing substrate and food.

Dustbathing is a natural behaviour in birds and has been observed in the ancestor of domestic hens, the red jungle fowl (e.g. Vestergaard *et al.*, 1990; Olssen and Keeling, 2005). The main function of dustbathing is to regulate and distribute feather lipids (van Liere and Bokma, 1987). This process maintains the feathers in good condition, and it is feather condition that may be a causation factor of dustbathing behaviour (van Liere, 1992). Dustbathing behaviour in laying hens occurs approximately every second day and peaks during the middle of the day (Vestergaard, 1982). The variation in the occurrence of dustbathing can be changed by a number of factors including the type of substrate on offer (van Liere *et al.*, 1990), the position of the dustbath within a cage (Petherick *et al.*, 1995) and environmental features such as temperature (Duncan *et al.*, 1998). In addition, hens have been found to prefer specific types of dustbathing substrate (Shields *et al.*, 2004; de Jong *et al.*, 2006). When deprived of dustbathing opportunity, hens have been found to increase the number of dustbathing sessions (Widowski and Duncan, 2000) and the duration of their dustbathing session when next allowed (Vestergaard, 1982), indicating an increase in motivation to dustbathe after a period of deprivation. Additionally, there has been evidence to suggest that dustbathing deprivation can cause an increase in hen plasma corticosterone concentrations (Vestergaard *et al.*, 1997), a sign of physiological stress.

The feeding behaviour of laying hens has been found to have a distinct diurnal pattern. Laying hens have been found to increase their feed intake late in the day (Savory, 1980). This is likely to occur in order to fill the crop at night (when no feeding occurs) and also to meet nutritional requirements during egg formation. There is also a minor peak in feed intake after oviposition, likely to be due to a reduction in intake during and prior to lay. Previous studies of domestic hens have found that feed deprivation increases exploratory behaviour (Nicol and Guilford, 1991) and decreases both the time taken to run through a Y-maze (Petherick *et al.*, 1993) and push a weighted door in order to obtain

food (Petherick and Rutter, 1990). These behavioural changes are interpreted to result from an increased motivation to find and consume food. Furthermore, deprivation of feed has been linked with increased frustration and stereotypic behaviour in hens (Duncan and Wood-Gush, 1972) and an increase in heterophil to lymphocyte ratios (Jones, 1989), which are signs of behavioural and physiological stress, respectively.

The aim of this study was to observe the effects of short-term deprivation of feed and dustbathing substrate on the motivation and preferences of mature laying hens in two Y-maze tests. The two tests examined the effects of deprivation by measuring the choice behaviour and speed of movement through the Y maze. Non-comparative Y maze tests (i.e. resource vs. nothing) were conducted to ensure that choice behaviour was simplified and to address the possibility that choice behaviour for one resource was not distracted by presence of another, especially food, as some authors have suggested (Petherick et al., 1993).

1.2 Methodology

1.2.1 Animals and housing

20 commercial laying hens (Hyline Brown Strain) were housed indoors in individual furnished cages (0.57m x 0.50m x 0.48m). The birds were 28 weeks of age at the commencement of the pre-experimental phase and had not been raised with a dustbathing substrate. Each cage contained a nest box, dustbath and a perch. Food was available *ad libitum* externally at the front of the cage in a feed trough and water was available *ad libitum* via a drinker at the rear of the cage. Hens were fed a commercial layer pellet feed. All birds had visual contact with other individuals as well as physical contact through mesh dividers between pairs of cages. The hens were kept in a constant environmental temperature (21°C) and had a light:dark cycle of 16:8 hours.

Prior to the first experiment one hen died of an unrelated cause (liver damage due to obesity). Additionally, one hen failed to move voluntarily in the Y-maze and was therefore removed from the study. Hence, 18 hens were used in this study.

1.2.2 Pre-experimental phase

For the first week after placing the hens into individual cages in the experimental facility, human contact was imposed twice a day over seven days. Initially the hens were gently touched, however over the week the intensity of interaction was increased and birds were eventually picked up and held for a short period (one to two min) and then placed back into the home cage.

1.2.3 The Y-maze apparatus

The Y-maze was a purpose-built apparatus constructed out of metal. The floor and removable roof pieces were constructed out of mesh, while the walls in the junction of arms (called the “choice area”) and arm gates were constructed out of solid metal (Figure 1). The start box had mesh walls and the gate between the start box and choice area was also mesh, 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 wall and roof mesh was a smaller gauge. 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.

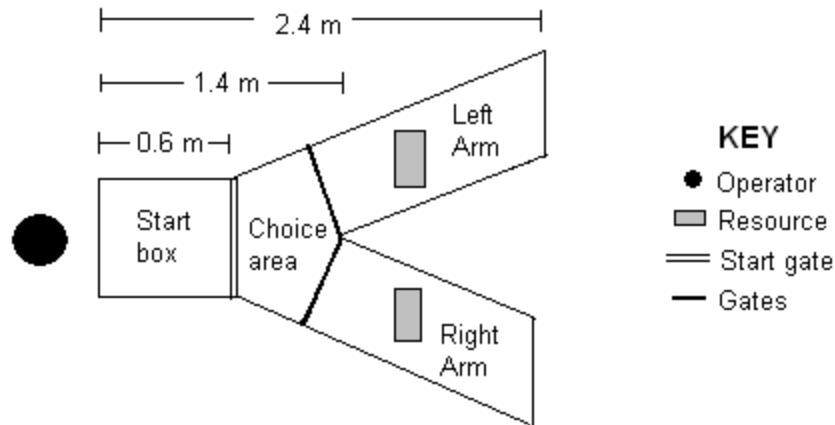


Figure 1. Diagram of the Y-maze apparatus

1.2.4 Procedure

This experiment was conducted in two parts: the first examined the effects of feed deprivation on choice behaviour for feed and the other examined the effects of deprivation of a dustbathing substrate on choice behaviour for a dustbathing substrate. Both Parts 1 and 2 consisted of three phases; familiarisation with the Y-maze, training and testing. All parts of the experiments were conducted from 11am to ensure birds were post-oviposition and thus eliminate any pre-laying behaviours, which may influence the hens behaviour in the Y-maze. If a hen was showing any signs of laying behaviour at the time of familiarisation, training or testing, that hen was run at the end of the scheduled session for that day.

1.2.4.1 Familiarisation

In a randomly determined order, each bird was individually placed for 10 s in the start box in the empty Y-maze with both arm gates open. The start box gate was then opened and the hen was given 30 s to enter the choice area. If the hen had not moved out after 30 s, she was gently pushed out of the start box. The bird was then given another 30 s to move into one of the Y-maze arms and cross the 'choice line' (position of the closed arm gate). If the hen had not moved within 30 s, she was gently pushed forward, towards the closer arm. After this, the hen was confined in the arm of choice (by closing the gate of the opposite arm) for a period of 2 min. This procedure occurred for three consecutive trials per bird on one day for each part of the experiment. There was only one day of familiarisation as the birds had previously been used in a study in this Y-maze, therefore they were somewhat familiar with the apparatus.

The familiarisation period was also used to determine any side preferences for individual birds. A side preference was defined as a bird consistently choosing one side of the Y-maze on all three familiarisation trials.

1.2.4.2 Training

Birds were placed individually in the Y-maze with the resource (a tray of feed, covered by metal mesh to prevent dustbathing for Part 1 and a tray of dustbathing substrate for Part 2) located in a randomly determined arm (left or right). If a bird was found to have a side preference in the familiarisation phase, the side at which the resource was placed was opposite to that of the preference. To ensure hens had experience both with and without the resource, each hen was exposed to one arm at a time (i.e. gate to the opposite arm was closed), with alternating exposure to each arm over four consecutive trials per day over three days. The side of first exposure was randomly determined for each bird.

For Part 1, all hens were deprived of feed for 30 min pre-trial to standardise feed intake immediately prior to trials for all birds and 30 min post-testing to ensure no associative learning could occur (i.e. birds could not learn an association between being returned to their home cage and the provision of feed). Hens had *ad libitum* access to their dustbath during the whole training phase in Part 2.

In a randomly chosen order each bird was individually placed in the start box of the Y-maze for 10 s, followed by 30 s after the start box was opened to enter the choice area. If she had not moved into the choice area, she was gently pushed out of the start box. Each hen was allowed 30 s to move into the available arm, and was gently pushed forward towards the open-gate arm if she had not moved forward in this time. This push in the choice area was referred to as a handler 'intervention'. If she had not entered the arm after a further 30 s, she was given another 'intervention'. This continued until the hen crossed the choice line into the arm, after which she was confined in the Y-maze for a period of two min.

1.2.4.3 Testing

Hens were randomly allocated into one of two treatments for each experiment. For Part 1, where feed was the resource under investigation, the treatments were:

- 0.5 h feed deprivation pre-testing ("control"),
- 4 h feed deprivation pre-testing ("deprived").

Dustbathing substrate was the focus for Part 2 and the treatments were:

- 0 h (i.e. *ad libitum* access) ("control")
- 22 h (mean, overnight deprivation) ("deprived"). Birds in this treatment group were allowed two hours of dust contact in their home cage post-testing each day (to avoid cumulative deprivation over the three testing days), then the dustbath was removed until the hen had completed the following days testing after which another two hours of contact was provided.

This was a cross-over designed study in which the deprivation treatment for each hen was alternated after the first testing phase for part of the experiment. This ensured all hens were tested under each treatment.

In a randomly chosen order, each hen was placed in the start box of the Y-maze. For both Part 1 and Part 2, the same resource tray was in the arm in which the bird had been 'trained' to associate the presence of the resource during the training phase. Both arm gates were open to allow the bird to choose an arm. After 10 s in the start box, the start box gate was opened. The hen was allowed 30 s to enter the choice area, and was gently pushed into the choice area if she had not moved. After leaving the start box, the start box gate was closed. The hen was given a further 30 s to move into an arm and was gently pushed forward towards the closer arm (again, referred to as an 'intervention') if she had not moved. This was repeated until the hen had crossed a choice line. The gate of the arm not chosen by the hen was then closed and the bird was confined in the Y-maze for two minutes. Each bird was tested on four consecutive trials per day over three days for each experiment.

The latency to leave the start box, the time to cross a choice line, the arm chosen and the number of interventions were manually recorded for each hen.

1.3 Statistical analysis

For both Part 1 and Part 2, analysis of variance (ANOVA) was used to determine the effect of deprived and control treatments, individual hen variation and the effect of the order of treatment (i.e. control first and then deprived or vice versa) on the resource choice, time to choice, latency to leave the start box and number of interventions. A Chi-square test was applied to the choice data to determine if the choice for feed was different to that of a random choice (i.e. 50:50). Additionally, the effect of trials over days was determined by constructing in Part 1 a two (treatments; "**Control**" (0.5 h pre-testing deprivation of feed) vs. "**Long**" (4 h pre-testing deprivation of feed)) by four (trials; 1,2,3,4) ANOVA

and in Part 2 a two (treatments; “Control” (no pre-testing deprivation of a dustbathing substrate) vs. “Long” (22 h pre-testing deprivation of a dustbathing substrate)) by four (trials; 1,2,3,4) ANOVA. All levels of statistical significance were set at P=0.05.

1.4 Results

1.4.1 Resource choice

1.4.1.1 Part 1

There was no significant difference between deprivation treatments in the proportion of trials in which hens chose the resource arm containing feed over the empty resource arm (P=0.54). However, the hens’ choice for feed was significantly different from a random choice (i.e. 50:50) for both control ($\chi^2_1 = 48.22$, P < 0.001) and deprived ($\chi^2_1 = 42.02$, P < 0.001) treatments (Table 1). There was no effect of the order of treatment imposed (P=0.937).

Table 1 Chi-square test for choice of feed over nothing in Part 1, Experiment 1

**Control-*

	Feed	Chance	Total
Feed	84.72	50.00	134.72
Nothing	15.28	50.00	65.28
Total	100.00	100.00	200.00

Degrees of freedom: 1, $\chi^2 = 48.22$, P < 0.001

* Control, 0.5 h pre-testing deprivation of feed; Long, 4 h pre-testing deprivation of feed.

**Long-*

	Feed	Chance	Total
Feed	82.41	50.00	132.41
Nothing	17.59	50.00	67.59
Total	100.00	100.00	200.00

Degrees of freedom: 1, $\chi^2 = 42.02$, P < 0.001

1.4.1.2 Part 2

Part 2, concentrating on dustbathing substrate, found that there was no significant difference between deprivation treatments in the proportion of trials in which hens chose the resource arm containing dust over the empty resource arm (P=0.089). The hens’ choice for dustbathing substrate was not different from a random choice (i.e. 50:50) for both control ($\chi^2_1 = 2.93$, 0.05 < P < 0.10) and deprived ($\chi^2_1 = 0.08$, 0.90 < P < 0.75) treatments (Table 2). Additionally, there was no effect due to the order of treatment (P=0.284).

Table 2 Chi-square test for choice of feed over nothing in Part 2, Experiment 1

Control-

	Feed	Chance	Total
Feed	58.56	50.00	108.56
Nothing	41.44	50.00	91.44
Total	100.00	100.00	200.00

Degrees of freedom: 1, $\chi^2 = 2.93$, P > 0.05

* Control, 0 h pre-testing deprivation of a dustbathing substrate; Long, 22 h pre-testing deprivation of a dustbathing substrate.

Long-

	Feed	Chance	Total
Feed	48.61	50.00	98.61
Nothing	51.39	50.00	101.39
Total	100.00	100.00	200.00

Degrees of freedom: 1, $\chi^2 = 0.08$, P > 0.05

1.4.2 Time to choice

1.4.2.1 Part 1

Hens were significantly faster moving through the choice area to a Y-maze arm when deprived of feed for the deprived treatment compared to control ($F_{1,17}=4.66$, $P=0.045$) (Figure 2).

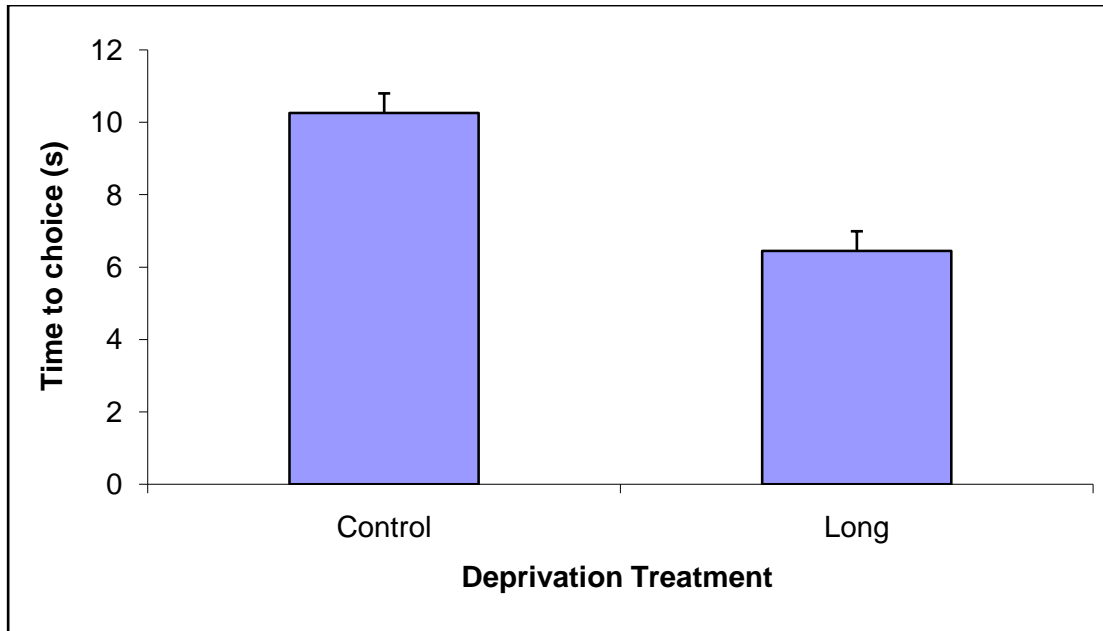


Figure 2 The mean time taken to move through the choice area to a Y-maze arm (i.e. "make a choice") for each deprivation treatment in Experiment 2. Letters indicate significant differences, $P<0.05$. Error bars show s.e.m.

However when only trials where the feed arm was chosen were included in this analysis, this difference was not statistically significant, although a strong tendency still remained ($P=0.052$). There was also a tendency ($P=0.056$) for hens to take an increasingly longer time to make a choice over each trial, each day in Part 1. The order of treatments had no effect on the time to choice ($P=0.456$).

1.4.2.2 Part 2

There was no effect of deprivation treatment on the time taken in all trials ($P=0.610$), or in trials where only the dustbath arm was chosen ($P=0.152$). However, in only trials where the dustbath arm was chosen (i.e. excluding the trials in which the empty arm was chosen), the time taken to move into the arm increased with each of the four trials over each day ($F_{3,222}=3.739$, $P=0.012$) (Figure 3). There was no effect due to the order of treatment ($P=0.393$).

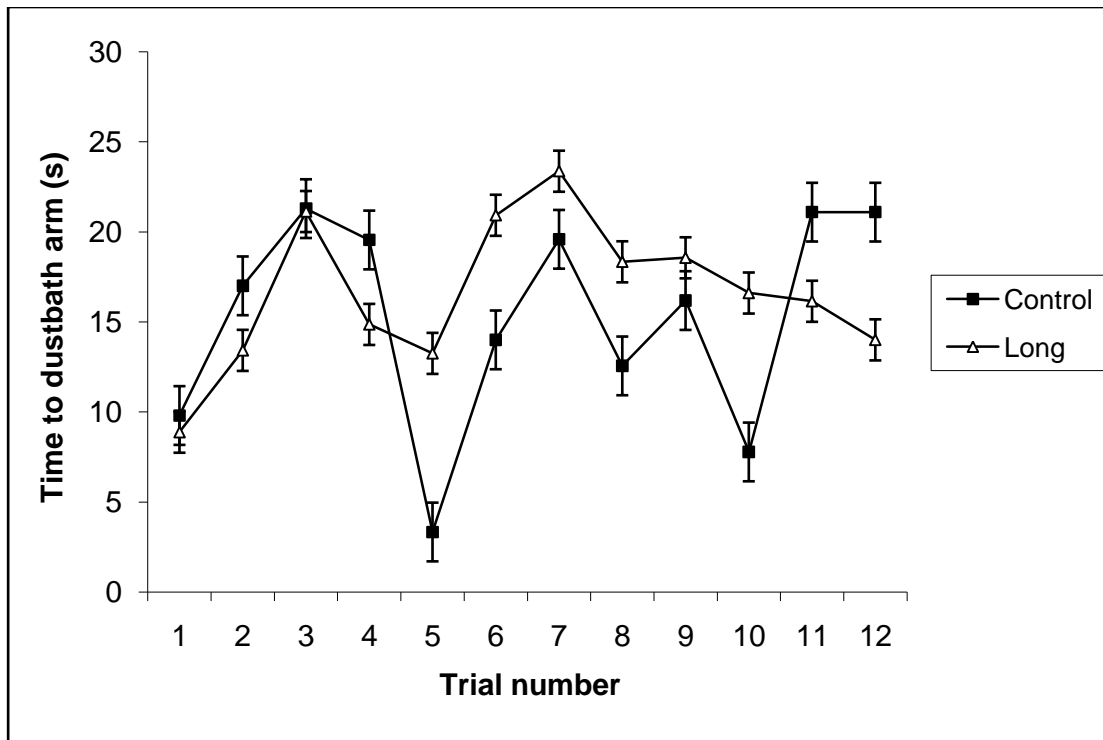


Figure 3 The mean time taken for hens to choose only the dustbath arm in Part 2, Experiment 1. The trial number refers to trials within all three days; day 1 (trials 1-4), day 2 (trials 5-8) and day 3 (trials 9-12). Error bars show s.e.m.

1.4.3 Latency to leave start box

1.4.3.1 Part 1

Hens were significantly faster to leave the start box (to enter the choice area) when under the deprived deprivation treatment compared to control ($F_{1,17}=6.23$, $P=0.023$). In addition, the latency to leave the start box increased with each of the four trials each day ($F_{3,420}=12.003$, $P<0.001$), with a similar pattern observed on each of the three days (Figure 4). There was no treatment/trial interaction ($P=0.877$) or any effect due to the order of treatments ($P=0.293$).

1.4.3.2 Part 2

The dust deprivation treatments had no effect on the latency to leave the start box ($P=0.228$). However, there was tendency for an increase in latency to leave the start box over each trial over the three days, similar to that observed in Part 1 ($P=0.053$). The order of treatments had no effect ($P=0.750$).

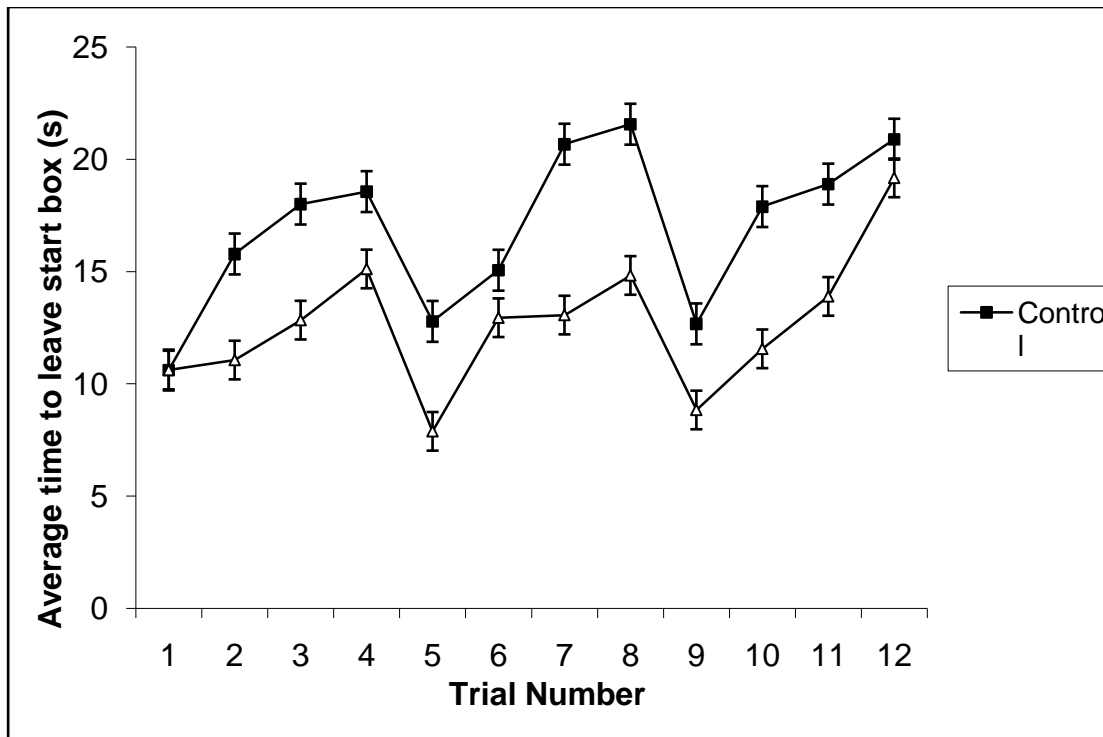


Figure 4 The mean time to leave the start box for Part 1, Experiment 1. The trial number refers to trials within all three days; day 1 (trials 1-4), day 2 (trials 5-8) and day 3 (trials 9-12). Error bars show s.e.m.

1.4.4 Number of interventions

1.4.4.1 Part 1

There was no significant difference between treatments in the proportion of trials that required at least one intervention in Part 1 ($P=0.41$). The order of treatments had no effect on the number of interventions ($P=0.512$).

1.4.4.2 Part 2

There was no significant difference between treatments in the proportion of trials that required at least one intervention in Part 2 ($P=0.92$). There was no effect due to the order of treatment ($P=0.455$).

1.5 Discussion

The hens moved faster when feed deprived in comparison to the control treatment. This result has been observed in other studies (Petherick *et al.*, 1992 (in an alleyway); Petherick *et al.*, 1993 (in a Y-maze)). Using the speed of movement as a measure of animal motivation has an advantage over other measurements as it requires the birds to undergo less training and learning than other techniques (e.g. operant conditioning) but still requires a hen to spend more energy (i.e. run faster) when highly motivated. However, the study by Petherick *et al.* (1992) found no difference in the speed of movement when hens were under deprivation treatments of 6, 12 and 18 hours, indicating that speed of movement may not differentiate between different degrees of deprivation. However, the authors suggest that this result been due to the start time of deprivation and its relation to feed intake in hens. For example, as hens do not consume food during their dark (night) period, if hens were deprived of

feed just prior to this period, it is likely this duration of feed deprivation would be similar to a deprivation period commencing the following morning.

There was no difference in the proportion of trials in which feed was chosen for both deprivation treatments in this study. The proportion of choices for feed under both deprivation treatments was high (around 83%) however animal preference tests rarely, if ever, produce results of 100% choice. The tendency for animals to not have 100% reliability in their choice is one criticism of animal preference tests. However, Duncan (1978) suggested that this might have some adaptive value for animals. Arnold (2005) went on to state that by sometimes choosing an alternative choice, the animal could thus observe if this minority choice has meaningfully changed since the animals' last visit. Alternatively, the time allocated in the Y maze to the hens over the four test trials may have allowed the hens to experience some satiety, leading to increased exploratory behaviour in the maze

The increased time taken to leave the start box over the daily trials suggests that the hens' motivation to move to the tray of feed declined with each trial over each day. This may be explained by a motivation model similar to that proposed by Hughes and Duncan (Hughes and Duncan, 1998) where internal and external factors influence the animal's motivational state. When the hens were feed deprived a number of factors may influence the animals' motivational state. These could include physiological and social factors as well as the frustration and stress of not being able to perform feeding behaviour. These factors would impact directly on the animal's motivational system. Once in the Y-maze these factors, though impacting the motivational state in relation to food, would cause the bird to leave the start box quickly and move to the arm containing food. Appetitive behaviour (i.e. searching for food) would produce a short-term increase in feeding motivation, which would decrease as feeding behaviour continues. Thus, once the bird had consumed some food in the first trial, her motivation to move as quickly out of the start box would be reduced as the bird would experience some satiety and be less motivated by physiological factors. As a similar pattern of latency to leave the start box over trials was observed in both treatments of feed deprivation (control and deprived), it could suggest that feeding behaviour is important to hens but that hens deprived of feed for a longer period moved out faster, suggesting a higher motivational state for feed in these birds.

Alternatively, the hens' exploratory behaviour may have caused the birds move out of the start box quicker initially due to the novelty of the situation. After each trial the novelty of the environment would decrease, leading to a longer time to leave the start box. However, this pattern of leaving the start box was not observed when the dustbathing substrate was presented.

The dustbathing substrate did not appear to be an attractive resource to these hens, as their choice behaviour when presented with this resource appeared to be random (as it was around 50% for dust). Petherick *et al.* (1993) also found a low proportion of Y-maze trials in which hens chose a dustbathing substrate and suggested that the experimental situation may have been inhibiting as many birds interacted with their dustbath on return to their home cage. The hens in this study were reared in cages with no experience of dustbath substrates however, even without early dustbath experience, hens have been shown to display dustbathing as an adult (Larson *et al.*, 2000). Casual observations of the hens over the five-week study period revealed only three hens displaying dustbathing behaviour. These hens may not have had sufficient exposure to the dustbath in their home cages to develop and engage in regular dustbathing behaviour and thus additional functional experience with the dustbath may have altered hens' choice behaviour.

Dustbathing behaviour may be socially influenced (Duncan *et al.*, 1998) and as hens were tested in the Y-maze individually, this may also have influenced their low choice for the dustbathing substrate. Another consideration is that dustbathing substrate may be used as a foraging substrate. Dawkins (1989) found that the red junglefowl (ancestor of the domestic hen) spent a significant amount of the day performing foraging behaviour. This may suggest that domestic hens too, might be motivated to perform these behaviours. However, Schutz and Jensen (2001) have shown that more domesticated breeds of poultry display less foraging behaviour than their undomesticated ancestor, and therefore hens may not be motivated to choose the dustbathing substrate for foraging behaviours either.

Finally, the three-day testing period may be an inadequate testing period for dustbathing substrate, as dustbathing behaviour occurs on average every second day (Vestergaard, 1982). In future research, this should be rectified by testing hens over a longer period and depriving hens of dustbathing substrate for a longer period to ensure there is a deprivation of dustbathing behaviour.

The major findings of this study are twofold. First, the hens' choice for feed was higher than that expected if the choice was random for both control and deprived treatments. Second, in contrast, the hens' choice for dustbathing substrate was not different from that expected if the choice was random, for both control and deprived treatments. These results are similar to Petherick *et al.* (1993) who also found hens chose feed significantly more often compared to a dustbathing substrate regardless of deprivation treatments, suggesting a high attractive value for feed. From an evolutionary perspective, food is a very important resource as it is a necessity for animal survival. Therefore, it is not surprising that hens chose this resource on the majority of trials.

The findings from this study indicate that laying hens are able to make choices in a Y-maze and short-term resource deprivation has no effect on the choice behaviour of laying hens for feed or dustbathing substrate. However, the deprivation treatment did have an effect on the feeding motivation of hens, indicated by an increase in speed of movement through the Y-maze when feed deprived.

Experiment 2: Measurement of resource preferences of laying hens in a Y maze choice test

2.1 Introduction

The housing of laying hens in conventional cages is internationally one of the most controversial animal welfare issues and has a high public profile. This negative public sentiment towards cages has led to major changes in the future in their design. For example, under European poultry welfare Directives, by 2012 cage layer systems must provide ‘furniture’, which includes a nest box, dust bath, perch and claw shorteners. Public interest in the welfare of hens housed in indoor systems is likely to increase and clearly science has an important role in understanding the welfare implications of these systems. From an animal welfare perspective, there is increasing interest in the measurement of the preferences of farm, laboratory and captive animals in relation to requirements within these environments. Preferences can be measured as a means to determine what resources are important to an animal and therefore important for maintaining and enhancing animal welfare. Initial use of preference methodologies appeared in the literature in the 1970s (e.g., Hughes and Black, 1973; Dawkins, 1976). These early investigations created much scientific debate relating to conceptual and methodological difficulties (e.g., Duncan, 1977; Dawkins, 1977; van Roojen, 1982). Nevertheless, preference testing using a Y maze apparatus that allows a choice between access to two different resources has been used to provide information about specific features in the animal environments such as flooring on raceways (Hutson, 1981), restraint methods (e.g., Pollard *et al.*, 1994), handling treatments (Rushen, 1986) and ramp design (Phillips *et al.*, 1988), with the overriding objective of optimising captive environments for animal inhabitants. Essentially, these tests are designed to answer the question “*what is the relative importance of this feature for this animal?*”.

One method of more closely assessing laying hen choices through preference testing is to experimentally alter motivational state by depriving hens of access to a resource (such as feed) and observing the impact of prior resource restriction on subsequent choice behaviour in a Y maze. Barnard, (2004) defines motivation as the internal decision making process an animal uses to make a choice about its behaviour. Thus, in terms of Y maze preference tests, if restriction of resource 1 leads to an increase in choice for that resource over time, but restriction of resource 2 does not increase choice for resource 2 over time, then it seems likely, in the context of the specific utility offered for each resource, that hens’ are less motivated to choose resource 2 and therefore, it may be less important to the hen than resource 1.

The present experiment measured the relative preferences of laying hens for feed, social contact and a dustbath substrate, using the described method of resource restriction to alter motivational states.

2.2 Methodology

2.2.1 Subjects and housing

Forty-eight commercial laying hens (*Gallus gallus domesticus*) were individually housed indoors in furnished cages (57cm x 50cm x 48cm) each containing a dustbath at floor level. The birds were divided into three groups of 16 birds for use in one of three separate experiments. The birds were 41, 48 and 54 weeks of age at the commencement of the pre-experimental phase for parts 1, 2 and 3 respectively, and had not been raised with dustbath access. Commercial layer pellets were available *ad libitum* externally at the front of the cage and water was available *ad libitum* via a drinker at the rear of the cage. All birds had auditory and olfactory contact with other birds and visual contact with neighbouring birds, as well as some limited physical contact through mesh dividers between pairs of

cages. The hens were kept in a constant environmental temperature of 21°C and a light:dark cycle of 16:8 hours.

2.2.2 Y-maze apparatus

The Y-maze was a purpose built apparatus with solid metal walls, removable mesh roofing and a floor constructed of mesh identical to flooring in the birds' home cages (see Figure 5). The gate between the start-box and choice area was also mesh, allowing visual contact with maze arms from the start-box. The gates between the choice area and each arm were solid metal. All gates were pulley operated and could be opened and closed by one operator standing in front of the start box.

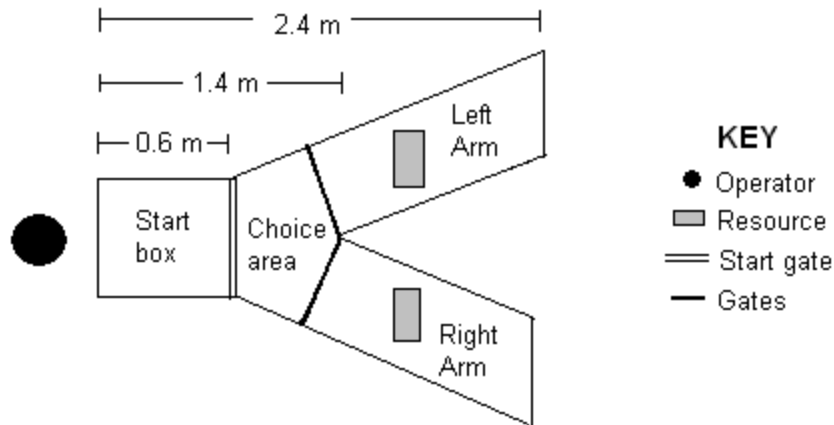


Figure 5 Diagram of a Y maze apparatus

2.2.3 Procedure

2.2.3.1 Pre-experimental phase

Birds were housed in the individual cages with dustbaths (containing sawdust) for three weeks prior to commencement of the experiment. During this period all birds were familiarised with human handling through incremental exposure to increasing intensity of human contact. Initially, over a period of three days each cage was opened and birds were exposed for 30 s per day to a handler moving a hand around in the cage. This was followed by five days of physically touching and stroking the birds on the back for 30 s per day, and finally picking the birds up and holding them for 30 secs per day for the seven days before the experiment commenced.

2.2.3.2 Familiarisation to the Y maze

All birds were exposed to the Y maze environment over three days with no resource in either maze arm. During this phase, the gates between the choice area and each arm were left open, however the start-box gate was initially closed. Each bird was carried from its home cage and placed in the start-box of the maze for 20 s before the start-box gate was opened. Birds were allowed 30 s to move out of the start-box before movement was gently encouraged by pushing the bird forward with a flat hand on the tail. Once in the choice area, the start-box gate was closed and birds were allowed another 30 s to move into a maze arm before movement was encouraged by gentle pushing. Once in an arm, access to the opposite maze arm was closed using the appropriate gate and birds were left in the maze for two minutes before being carried back to the start-box for the next trial (or to the home cage when the sequence of trials was complete). Each bird was exposed to the maze in this way for three consecutive trials each day over the three days, and birds were run in a different random order each day. The two minute period of confinement with the chosen resource was selected based on the results of a pilot

study which measured the amount of time spent interacting, in the first bout, with feed and dustbath resources, respectively, in a test cage.

2.2.3.3 Training phase

During the training phase, birds were deprived of feed and dustbath access for 30 minutes prior to each Y maze training session in order to standardise exposure to these resources. A comparable removal of access to social contact was not practicable in the housing arrangement.

Training took place over 2 days with 4 consecutive trials per bird per day. In each trial only one arm of the maze was available, with the other closed off by the appropriate gate between choice area and arm. The available maze arm was alternated across the 4 trials each day so that each bird was exposed to each arm twice per day.

Resource 1 was presented in one maze arm and Resource 2 in the other (see Table 2 for resource descriptions). Pairing of maze arm and resource was randomly assigned to each bird and the pairing remained consistent for each bird within each experiment.

The procedure for each trial was identical to that described in the familiarisation phase (however, once birds entered the available maze arm there was no need to shut the opposite gate as it was already closed). Time taken to move out of the start-box and choice area, and number of pushes required to move birds out of the choice area were recorded. As with familiarisation, bird order was randomised each day.

2.2.3.4 Testing phase

In each of the three parts of this experiment, the effects of restrictions (see Table 3) of the two resources of interest on preferences were studied in a 2x2 factorial design over 12 days such that 4 birds were deprived of both resources, 4 were deprived of resource 1 only, 4 deprived of resource 2 only, and 4 were not deprived of either resource. The description of the two resources and their restrictions studied in each part are as follows:

Table 3 Description of resource and restriction treatments in Experiment 2

Resource	Description	Restriction treatment
Feed	Commercial layer pellets	Feed removed 3 hrs prior to testing
Dustbath Substrate	Fine-grain sawdust in 46.6 x 22.6 x 3.2 cm plastic tray	Dustbath tray was removed from cage 12 hours prior to first day of testing until after the last day of testing
Social Contact	Visual contact with neighbouring birds	Mesh divisions between cages replaced with solid partitions from 12 hours prior to first day of testing until after the last day of testing

Part 1. The two main effects studied were feed at 2 levels, (1) feed available in feed tray of the home cage ('Unrestricted') and (2) access to feed was removed from the home cage for 3 hours prior to first trial each day by covering the external feed trough in front of the cage ('Restricted') and dustbathing substrate ('dust') at 2 levels, (1) dust in a dustbathing tray was available in the home cage ('unrestricted') and (2) the dustbath tray was removed from home cage on the night before day 1 of testing was not replaced until the end of the experiment ('restricted').

Part 2. The two main effects were dustbathing substrate ('dust') at 2 levels, as described for Part 1, and social contact at 2 levels, (1) visual contact with neighbouring birds at all times while in the home cage ('unrestricted') and (2) visual contact with neighbouring birds removed on the day before day 1 of testing by covering cage divisions with solid rubber partitions that remained in place until the end of the experiment ('restricted').

Part 3. The two main effect were feed at 2 levels, as described in Part 1, and social contact at 2 levels, as described in Part 2.

Birds that were not restricted of feed or dust in the three experiments had feed and dustbath removed for 30 min prior to testing, as in the training phase in order to standardise contact with these resources immediately prior to testing.

Testing was conducted over 12 days and consisted of 2 trials per bird per day. Again, bird order was randomly generated for each day of testing. Both maze arms were available on every trial, thus birds were able to move into either arm from the choice area. Birds that failed to move after 30 s in the start box and/or after 30 s choice area were gently pushed as described in the familiarisation phase. Identical maze arm and resource pairings were maintained for each bird consistent with those used in the training phase for each part. The procedure for each trial was identical to that described for the familiarisation phase, and once a maze arm was entered, access to the opposite arm was closed using the appropriate gate.

Times taken to leave the start-box and choice area, number of pushes required to leave the choice area and resource chosen were recorded for each trial.

2.3 Statistical analysis

For each part of the experiment a two factorial analysis of variance was used to determine treatment effects of resource 1 restriction and resource 2 restriction, and the interactions of these factors, on choice of resource and time taken to move out of the start box(s), time taken to move into a maze arm (s) and number of pushes required to move birds out of the choice area after 30 s. Binomial tests were conducted to determine the overall number of birds' whose resource choices differed from random (i.e. 50 %) for each part. A one-way analysis of variance was conducted for parts 1, 2 and 3 to determine whether there was an effect of resource type chosen on time to move into a maze arm.

2.4 Results

2.4.1 Part 1 – Feed vs. Dust

There were a total of 24 trials during the testing phase. The overall proportion of feed choices by hens was 78 % of trials, with a binomial test indicating that 11 of the 16 birds chose feed on significantly more than 50 % of trials ($P < 0.01$). The time taken to move into a maze arm from the choice area was greater ($P < 0.01$) when dust was chosen than when feed was chosen (Table 4).

Table 4 Mean times to move into maze arm for trials in which resource 1 and 2 were chosen for each part in Experiment 2. P value and LSD for each one-way Analysis of Variance presented.

	Resource 1 time to maze arm (s)	Resource 2 time to maze arm (s)	P value	LSD (P = 0.05)
Part 1	Dust	Feed		
	6.59	3.61	<0.01	1.423
Part 2	Dust	Social Contact		
	6.50	9.00	0.023	2.153
Part 3	Feed	Social Contact		
	4.03	7.75	<0.01	1.844

The proportion of trials in which feed was chosen was calculated for each bird and analysed for treatment effects. No effects of either feed (P = 0.140) or dust (P = 0.699) restriction were found in the analysis of all 24 trials (Table 5), and there was no interaction effect (P = 0.699). However, when only first trials of each day (total of 12) were included in the analysis, a significant (P = 0.027) effect of feed restriction was revealed (Table 5), with no interaction effect (P = 0.233). Birds that were feed restricted chose feed in the Y maze on a greater proportion of these first trials than birds that were not restricted.

Table 5 Proportion of Y maze trials in which feed was chosen for hens restricted (n=8) and not restricted (n=8) of feed and hens restricted (n=8) and not restricted (n=8) of dust for all trials and for first trials of each day only in Part 1 of Experiment 2

	Restricted	Unrestricted	P value	LSD
<i>All trials</i>				
Feed	0.84 <i>(0.86)</i>	0.72 <i>(0.73)</i>	0.140 <i>(0.126)</i>	0.158 <i>(0.160)</i>
Dust	0.77 <i>(0.78)</i>	0.80 <i>(0.81)</i>	0.699 <i>(0.758)</i>	0.158 <i>(0.160)</i>
<i>First trials of each day only</i>				
Feed	0.91 <i>(0.92)</i>	0.70 <i>(0.73)</i>	0.027 <i>(0.034)</i>	0.166 <i>(0.168)</i>
Dust	0.78 <i>(0.79)</i>	0.82 <i>(0.84)</i>	0.624 <i>(0.562)</i>	0.166 <i>(0.168)</i>

Data excluding trials where pushes were required are presented in parentheses and italics.

When only trials where no pushes were required to move birds through the maze were included in the analyses, a similar pattern of results was observed (Table 5), with a significant increase (P = 0.034) in choice of feed by feed restricted birds in first trials of each day compared to birds that were not feed restricted.

The mean time taken to move out of the start box once the gate had been lifted, mean time to move out of choice area and number of pushes required were separately analysed for treatment effects (Table 6).

Although no significant treatment effects were revealed on any of these measurements, there was a trend ($P = 0.092$) indicating a decrease in time taken to move out of the start box in feed restricted hens compared to those that were not feed restricted.

Table 6 Mean latency to leave start box, latency to enter the maze arm and number of pushes required for hens restricted (n=8) and not restricted (n=8) of feed and hens d restricted (n=8) and not restricted (n=8) of dust for all trials in Part 1 of Experiment 2

	Restricted	Unrestricted	P value	LSD
<i>Latency to leave start box (s)</i>				
Feed	3.58	9.00	0.092	5.908
Dust	5.69	6.88	0.694	5.908
<i>Latency to enter maze arm (s)</i>				
Feed	3.56	4.98	0.260	2.410
Dust	4.27	4.26	0.993	2.410
<i>No. of pushes required</i>				
Feed	0.04	0.13	0.347	0.170
Dust	0.04	0.13	0.347	0.170

2.4.2 Part 2 – Dust vs. Social Contact

The overall proportion of dust choices was 46 % with 14 out of 16 birds' choice not differing significantly from random ($P = 0.645$). The overall speed of movement out of the choice area was greater ($P < 0.05$) when dust was chosen than when social contact was chosen (see Table 4 in previous section).

Analysis of the proportion of trials (out of a total of 24) in which dust was chosen in Part 2 revealed a significant effect of dust restriction (Table 7). Birds that were restricted of dust chose dust on a greater proportion ($P = 0.048$) of trials compared to birds that were not restricted. There was no effect of social contact restriction ($P = 0.886$) and no interaction effect ($P = 0.886$). When only first trials of each day (total of 12) were included in the analysis, no significant treatment effects were found for either dust ($P = 0.123$) or social contact ($P = 1.000$) restriction and there was no interaction effect ($P = 1.00$).

When re-analysed excluding trials where pushes were required to move birds out the of choice area, a similar pattern of results were observed except that the significant effect of dust restriction on dust choice ($P = 0.022$) was confined to the first trials of each day only (Table 7). There was also a tendency ($P = 0.062$) for choice of social contact to increase with restriction of social contact when trials with pushes were excluded.

There were no significant ($P > 0.05$) treatment effects observed on time taken to move out of the start box, time to leave choice area, or number of pushes required (Table 8). However, there was a trend towards social contact restricted birds moving faster into the maze arms ($P = 0.065$) and requiring less pushes ($P = 0.065$) compared to those with unrestricted social contact. There was also a tendency for less pushes required for dust restricted birds compared to those not restricted of dust ($P = 0.090$).

Table 7 Proportion of Y maze trials in which dust was chosen for hens restricted (n=8) and not restricted (n=8) of social contact and hens restricted (n=8) and not restricted (n=8) of dust for all trials and for first trials of each day only in Part 2 of Experiment 2

	Restricted	Unrestricted	P value	LSD
<i>All trials</i>				
Social Contact	0.45 <i>(0.46)</i>	0.46 <i>(0.39)</i>	0.886 <i>(0.062)</i>	0.142 <i>(0.150)</i>
Dust	0.53 <i>(0.46)</i>	0.38 <i>(0.47)</i>	0.048 <i>(0.892)</i>	0.142 <i>(0.150)</i>
<i>First trials of each day only</i>				
Social Contact	0.49 <i>(0.44)</i>	0.49 <i>(0.35)</i>	1.000 <i>(0.250)</i>	0.176 <i>(0.162)</i>
Dust	0.56 <i>(0.50)</i>	0.42 <i>(0.29)</i>	0.123 (0.022)	0.176 <i>(0.162)</i>

Data excluding trials where pushes were required are presented in parentheses and italics.

Table 8 Mean latency to leave start box, latency to enter the maze arm and number of pushes required for hens restricted (n=8) and not restricted (n=8) of social contact and hens restricted (n=8) and not restricted (n=8) of dust for all trials in Part 2 of Experiment 2

	Restricted	Unrestricted	P value	LSD
<i>Latency to leave start box (s)</i>				
Dust	10.57	13.00	0.334	4.836
Social Contact	9.67	13.91	0.105	4.836
<i>Latency to enter maze arm (s)</i>				
Dust	5.71	10.02	0.138	5.422
Social Contact	5.12	10.62	0.065	5.422
<i>No. of pushes required</i>				
Dust	0.13	0.46	0.065	0.328
Social Contact	0.15	0.45	0.090	0.328

2.4.3 Part 3 – Feed vs. Social Contact

The overall proportion of feed choices was 88 %, with 14 out of the 16 birds choosing feed on significantly more than 50 % of trials ($P < 0.001$). The overall speed of movement out of the choice area was greater ($P < 0.01$) when feed was chosen than when social contact was chosen (see Table 4 in previous section).

There were no significant effects of either feed ($P = 0.634$) or social contact ($P = 0.327$) restriction on the proportion of trials (out of a total of 24) in which feed was chosen (Table 9) and there was no

interaction effect ($P = 0.615$). Similarly, when only the first trials of each day were included in the analysis, no significant effects of either treatment were found (Table 9) and there was no interaction effect ($P = 0.615$).

Table 9 Proportion of Y maze trials in which feed was chosen for hens restricted (n=8) and not restricted (n=8) of feed and hens restricted (n=8) and not restricted (n=8) of social contact for all trials and for first trials of each day only in Part 3 of Experiment 2

	Restricted	Unrestricted	P value	LSD
<i>All trials</i>				
Feed	0.79 <i>(0.80)</i>	0.85 <i>(0.87)</i>	0.634 <i>(0.571)</i>	0.234 <i>(0.232)</i>
Social Contact	0.76 <i>(0.78)</i>	0.88 <i>(0.90)</i>	0.327 <i>(0.323)</i>	0.234 <i>(0.232)</i>
<i>First trials of each day only</i>				
Feed	0.82 <i>(0.82)</i>	0.90 <i>(0.90)</i>	0.615 <i>(0.544)</i>	0.014 <i>(0.234)</i>
Social Contact	0.81 <i>(0.81)</i>	0.91 <i>(0.91)</i>	0.406 <i>(0.438)</i>	0.014 <i>(0.234)</i>

Data excluding trials where pushes were required are presented in parentheses and italics

When these data were re-analysed excluding trials where one or more pushes were required to move birds out of the choice area, a similar pattern of results was observed with no significant treatment effects revealed (Table 9).

There were no significant treatment effects on time taken to move out of the start box, time to leave choice area, or number of pushes required (Table 10).

Table 10 Mean latency to leave start box, latency to enter the maze arm and number of pushes required for hens restricted (n=8) and not restricted (n=8) of feed and hens restricted (n=8) and not restricted (n=8) of social contact for all trials in Part 3 of Experiment 2

	Restricted	Unrestricted	P value	LSD
<i>Latency to leave start box (s)</i>				
Feed	7.32	5.58	0.506	5.086
Social Contact	6.25	6.64	0.881	5.086
<i>Latency to enter maze arm (s)</i>				
Feed	5.59	3.83	0.373	3.810
Social Contact	3.91	5.51	0.419	3.810
<i>No. of pushes required</i>				
Feed	0.83	0.63	0.708	0.108
Social Contact	0.78	0.68	0.851	0.108

2.5 Discussion

The main findings indicate that birds consistently chose feed over dust or social contact, irrespective of restriction of any of these resources in Part 1 and 3. The proportion of choices for feed in each part of this experiment was significantly greater than that for a random choice pattern (i.e. 50/50%).

In general, the overall choice for dust and social contact in Part 2 was similar (46 vs. 54% for dust and social contact, respectively), however restriction of dust but not social contact increased choice for the restricted resource (53% vs. 38% dust choices for dust restricted and unrestricted, respectively and 45% vs. 46% sawdust choices for social restricted and unrestricted, respectively), suggesting that dust restriction increased birds' motivation to access the dustbath substrate. Since the overall choice for dust or social contact in Part 2 was not significantly different from random (i.e. 50%), the result of an effect of dust restriction but not social contact restriction cannot be attributed to any difference in preference for dust over social contact. It is important to note that the levels of restriction studied in this experiment were arbitrary, with the utility (i.e. reward value) of the resource for restricted birds likely to differ between resources, and thus any interpretation of the relative attractiveness of the resources based on these arbitrary restrictions must be made cautiously. It is clear from the results of this experiment that birds consistently chose feed over dust or social contact, irrespective of restriction of any of these resources. This result that the preference for a biological necessity such as food overrides that of social or dustbathing preferences, makes evolutionary sense and is in agreement with other preference methodologies which have indicated the value of a feed resource exceeds those of other resources examined, and is often assumed to demonstrate the maximal response or preference in most cases (e.g. Dawkins, 1983).

The results of Part 1 are also consistent with Petherick et al. (1993), in which hens chose feed over a sand dustbathing substrate in a Y maze task when feed deprived but did not increase choices for sand over feed when deprived of sand. It should be noted that the resource utility of both feed and dustbathing substrate in Petherick et al (1993) most likely differed from that in the current study. Firstly, time allowed with the chosen resource differed between experiments; birds' in Petherick et al. (1993) were allowed 1 minute of contact with the chosen resource on the first 3 trials and then 30 minutes of contact on the last trial of each weekly test session. Secondly, different dustbathing substrates were used; sand vs. sawdust. Both the duration of interaction and the type of substrate may affect perceived resource value by birds in these studies.

Petherick et al. (1993) reported an effect of resource deprivation on speed of movement into the maze arm, where birds were faster when feed deprived than when sand deprived. In contrast, the speed of movement data from the current experiment are equivocal. There was a trend ($P = 0.092$) towards faster movement in the maze out of the choice area, but not out of the start box, by feed restricted hens in Experiment 1 (feed vs. dust) than unrestricted hens, but there was no evidence of faster movement in feed restricted hens in Experiment 3 (feed vs. social contact). Further, there were tendencies for hens that were social contact or dust restricted to move faster and require less pushes by the handler in Experiment 2 (dust vs. social contact) than unrestricted birds. It is somewhat surprising that no significant effects of restriction were found on speed of movement in the maze, since similar relationships have been reported by both Petherick et al. (1992, in an alleyway) and Petherick et al. (1993, in a Y maze) and by Laine et al. (2007, using the same breed of bird and apparatus, and a similar method to the current study). However, speed of movement may be affected by a number of other factors, such as competing motivations (e.g. fear, exploration), and bird age. In Petherick et al. (1992) and Petherick et al. (1993), birds were less than 20 weeks old, and in Laine et al. (2007), birds were 28 weeks; however in the current study, birds were 41 (Experiment 1) to 54 (Experiment 3) weeks. This age difference may partially explain the lack of an effect of restriction on speed of movement observed in the current study. Channing et al. (2001) reported both decreased activity in older compared with younger birds, and less time spent feeding in older birds (possibly indicative of a reduced feeding motivation in older compared with younger birds).

Interestingly, despite the lack of effect of resource restriction on speed of movement, there was evidence to suggest that speed was dependent on the resource chosen. In Parts 1 and 3, birds were

quicker to make feed choices than choices for the alternative resource, supporting the notion that birds had a higher motivation for feed than dust and social contact. In Part 2, birds were quicker to make dust choices than social contact choices, possibly indicating a higher motivation for dust than social contact, despite no evidence of a preference for dust over social contact in this comparison. Nevertheless, it is possible that variation in speed of movement reflected variation in motivation to access the resource in the Y maze, consistent with the finding of an effect of dust restriction on motivation to access dust.

Lack of voluntary bird movement in the current study may have interfered with accurate measurement of choice. Physically pushing birds that did not move after the maximum time allocated for a choice to occur, may have interfered with their choice behaviour. However, when trials where pushes were used were removed from the analyses, the main effects did not change, except for dust choice in Part 2; the increase in dust choice in dust restricted compared to unrestricted birds disappeared when trials with pushes were removed, although a significant effect of dust restriction was still present on first trials only, suggesting that possibly one 2 min exposure to dust may have satisfied dust bathing motivation enough to allow competing motivations, such as social contact or exploration, to take priority.

It is interesting to note that the finding of an effect of dust restriction on dust choice over social contact differs somewhat from the lack of any effect of dust restriction reported by Petherick et al. (1993) on dust choice over feed and by Arnold et al. (2007) on dust choice over an empty arm. The differences between these experiments suggest that the resource of comparison in a Y maze test most likely influences the comparative resource utility and therefore choice behaviour, along with other resource attributes such as resource type and duration of access to resource, as discussed above.

The difference in effect of dust restriction on dust choice between the current experiment and Arnold et al (2007) is somewhat surprising considering there was no experimentally manipulated competing resource assigned in the latter, presumably allowing even a moderate motivation to dustbath to be expressed. One explanation for this difference is the impact of experience on dustbathing motivation. Expression of dustbathing behaviour in laying hens can be affected by degree of prior exposure to dustbath substrates (e.g. Petherick et al., 1995; Larsen, Vestergaard & Hogan, 2000; Olsson et al., 2002). Although this phenomenon is generally reported in relation to the impact of experience during rearing, recent experience may also play a role in motivation to seek a dustbath substrate. In Arnold et al. (2007) access to dustbaths in the birds' home cages was relatively arduous (dustbaths were placed above a nestbox, and accessed via a perch), compared with the current study, where dustbaths were placed on the floor of the home cages, enabling easier access. Thus differences in the accessibility of dustbaths may have led to differences in familiarity with the dustbath substrate, and establishment of dustbathing routines in the days leading up to the Y maze tests. Future research investigating the administration of dustbaths in caged systems should identify or control for the impact of dustbath location on utilisation and subsequent dustbathing preferences.

In general, the results of all three parts in this study were in agreement, indicating a strong relative preference for feed over dust and social contact in laying hens. Agreement amongst the experiments and with other preference methodologies also suggests that the method used is likely to reliably measure laying hen choices under the conditions imposed in the present study. However, in order to be confident that the outcomes of these preference tests reflect the welfare requirements of the animal, additional evidence, particularly on the occurrence of abnormal behaviour, stress physiology and health when restricted of the resource or the behaviour of interest, are necessary to provide a more comprehensive assessment of the impact of restricting a resource or behaviour on welfare (Widowski and Hemsworth, 2008).

Experiment 3: The effect of quantity of reward on the choice behaviour of laying hens for dust and social contact

3.1 Introduction

While welfare-related animal preference tests have been conducted since the 1970's, many aspects of the design of preference tests continue to be arbitrary, as authors often fail to describe why or how certain aspects of their design were chosen. Examples of such factors include the number of test trials, the frequency of testing, the quantity of reward and the quality of reward (Fraser and Matthews, 1997). It is currently unknown whether factors such as these have an impact on the preferences of animals. This may potentially lead to spurious results. Research examining these arbitrary aspects of preference test design may well have important implications for the future design of preference tests. A well-developed methodology will allow scientists to study the preferences of animals and determine the environmental requirements of an animal. Potentially, these requirements may then be incorporated into livestock housing to optimise animal welfare.

The reward size an animal receives in a Y-maze preference test refers to the period for which the animal is confined within the chosen Y-maze arm following the choice made by the animal i.e., the amount of time the animal is allowed contact with the resource. This amount of time could potentially affect the choice behaviour of the animal on a subsequent trial, as this time could affect the attractiveness of the resource to the animal. For example, Kirkden and Pajor (2006) suggested that restricting the quantity of reward size might devalue some resources. To avoid this, some researchers have suggested allowing experimental animals to control the period for which they have access to a resource (Mason *et al.*, 1998). It has also been suggested, "the *value* of the environment (in an animal preference test) may be significantly affected by the period of time allowed" (Nicol, 1997).

Despite these claims, there has been no consensus on the reward size in Y-maze tests and this value in many studies appears to be arbitrary, as numerous studies do not state their reasoning behind the reward size chosen (e.g. Hughes, 1976; Dawkins, 1978, 1983; Lingberg and Nicol, 1996). This may suggest that this period has been chosen more for experimenter convenience rather than animal requirements with the resource. Previous Y-maze studies of laying hens have used a range of reward sizes. Early environmental preference studies by Dawkins (1977, 1981, 1982) used a reward size of 5 min. The reasoning behind this reward size was stated simply as to "ensure that the hen had a standard and measured amount of time in the environment of her choice" (Dawkins, 1977). Other hen Y- or T-maze studies have used 10 minutes (social contact, Lingberg & Nicol, 1996), 2 minutes (spontaneous alternation behaviour in an empty Y-maze, Haskell *et al.*, 1998) or even varied amounts of time (e.g. 3 minutes for each trial except for final trial for which there was 30 minutes to allow for dustbathing behaviour, Petherick *et al.*, 1993).

The aim of this study was to examine the effect of reward size on the choice behaviour of laying hens given a choice of dust and social contact in a Y-maze.

3.2 Materials and methods

3.2.1 Animals and housing

Thirty hens (*Gallus gallus domesticus*, Hy-line Brown laying strain) were chosen from 78 hens group-housed in cages (1.14 m x 0.50 m x 0.45 m). At 16 weeks the birds were slightly beak trimmed. The hens were moved to the experimental facility at approximately 20 weeks of age where they were placed in group cages with 6-8 birds per cage. When the hens were 27 weeks old, a dustbath (plastic tray, 0.46 m x 0.22 m 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 when they were 32 weeks of age. Hens were selected on their presumed social status, defined simply as either 'dominant' or 'subordinate', which was assessed during 5-minute direct observations of each cage when feed (a commercial layer pellet feed) access was provided following overnight feed deprivation (mean = 18 hours deprivation). Feed deprivation was carried out by covering each cage's feed trough with two metal covers. When each cage was re-allowed feed access, only one metal cover was removed thus reducing the space of the feed trough which subsequently did not provide enough space for all birds in the cage to feed simultaneously. Observations were made each morning, commencing at 1100 hours, over five consecutive days. Individual hens were identified via coloured leg bands. Each cage was observed for interactions between individuals and the willingness of individuals to gain access to feed, as well as other behaviours (e.g. pacing, vocalization, pecks between individual birds at the feed trough etc.).

Hens that were chosen as either dominant or subordinate 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 1-2 nipple drinkers at the rear. Hens had *ad libitum* feed and water in their home cage throughout the whole experiment. Each cage also contained a plastic dustbath (0.46 m x 0.22 m x 0.03 m) identical to the dustbath that was in the original group cages. The dustbath was filled daily with approximately 400 mL peat moss. Peat moss was chosen as the dustbathing substrate as it has been found to be a preferred substrate by laying hens (Petherick and Duncan, 1989; de Jong *et al.*, 2006). Cages were such that pairs of cages were separated by metal mesh, which allowed limited tactile contact between neighbours. These pairs of cages were separated from others by solid metal walls (see Figure 6). Each hen duo (i.e. a dominant and a subordinate hen chosen from the same group-housed cage) was randomly allocated into a pair of cages.

As dominant hens were chosen to be the test subjects for this experiment, they will be referred to as test hens from here onwards and the subordinate neighbouring hens will be referred to as non-test hens.

Hens were inspected, fed and peat moss 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 21°C and a light:dark cycle of 12:12 h, with lights coming on at 0700 h.

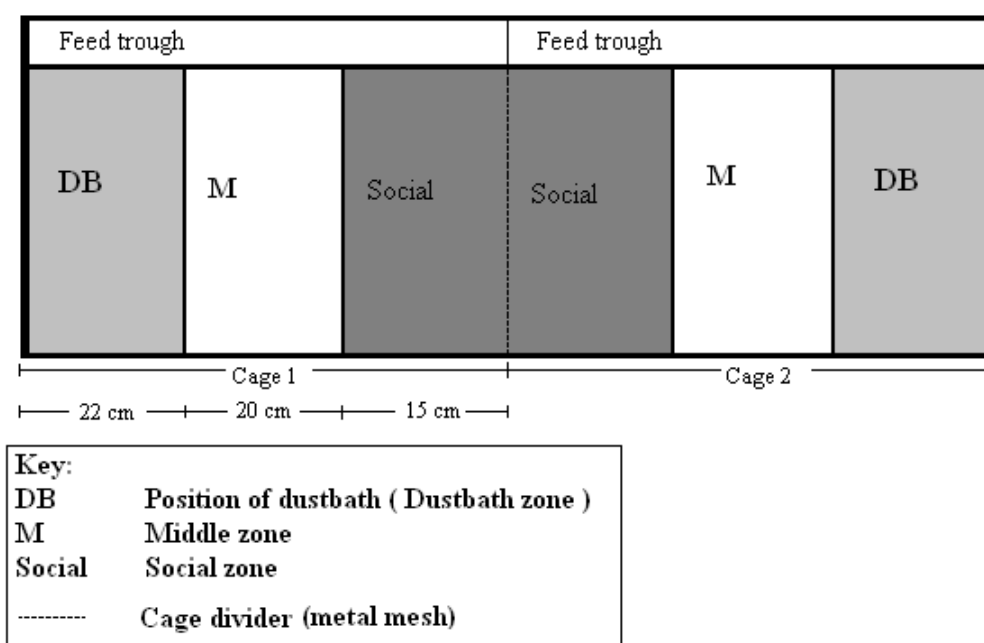


Figure 6 Diagram of a pair of hen cages and zones (not to scale).

3.2.2 Pre-experimental observations

Two weeks after the hens had been moved into their individual cages, hens were continuously digitally video recorded for 12 days. Each cage was recorded with its own camera and both cages in each pair (i.e. both the test and non-test hen) were recorded. Videos were observed and behaviours (Table 11) recorded.

3.2.3 Pre-experimental handling

To ensure hens were accustomed to being handled, all hens (including non-test birds) were given 1 minute of handling twice a day for 7 days. 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.

Table 11 Description of behaviours recorded during the pre-experimental period

Behaviour	Definition	Notes
Dustbathing	Commenced when the hen rolled on to her side and ended when she stood up and shakes or when she stood up and performs another behaviour for more than 5 mins. If the hen shook within 5 mins of standing up: - the end of the bout was at the time of the shake if there was no other behaviour in between. - the end of the bout was the time the hen stood up if she performed another behaviour before shaking	The incidence and duration (s) of dustbathing bouts was recorded
Dustbathing - following the dustbath being refilled	The occurrence of dustbathing during a one hour period following the hens' dustbath being refilled with peat moss was recorded. The same definition of dustbathing (as above) was used.	The occurrence and latency (s) to dustbathe in this hour was recorded. In addition, whether the dustbathing bout was real (i.e. in the dustbath) or sham (i.e. on wire) was recorded
Foraging	The number of pecks the hen directed at the dust/dustbath was recorded	
Social	The duration (s) that the test hen's head was in the social zone of her home cage was recorded	

3.2.4 Runway test

3.2.4.1 Runway apparatus

The main raceway in the runway apparatus was 1.72 m long and 0.6 m wide. It contained a compartment at each end (each 0.5 m long), which was separated to the main raceway by a metal mesh divider (Figure 7). The walls of the runway were solid metal and the floor and removable roof pieces were metal mesh. The main raceway of the runway had three removable roof pieces (each 0.57 m long). The area directly under each roof piece was defined to be a runway zone. Two cameras were mounted approximately 1.3 m above the runway. The cameras were positioned so that each one was directly above each edge of the centre roof piece so that when the hen moved from the centre zone to one of the other zones, it could easily and precisely be recorded.

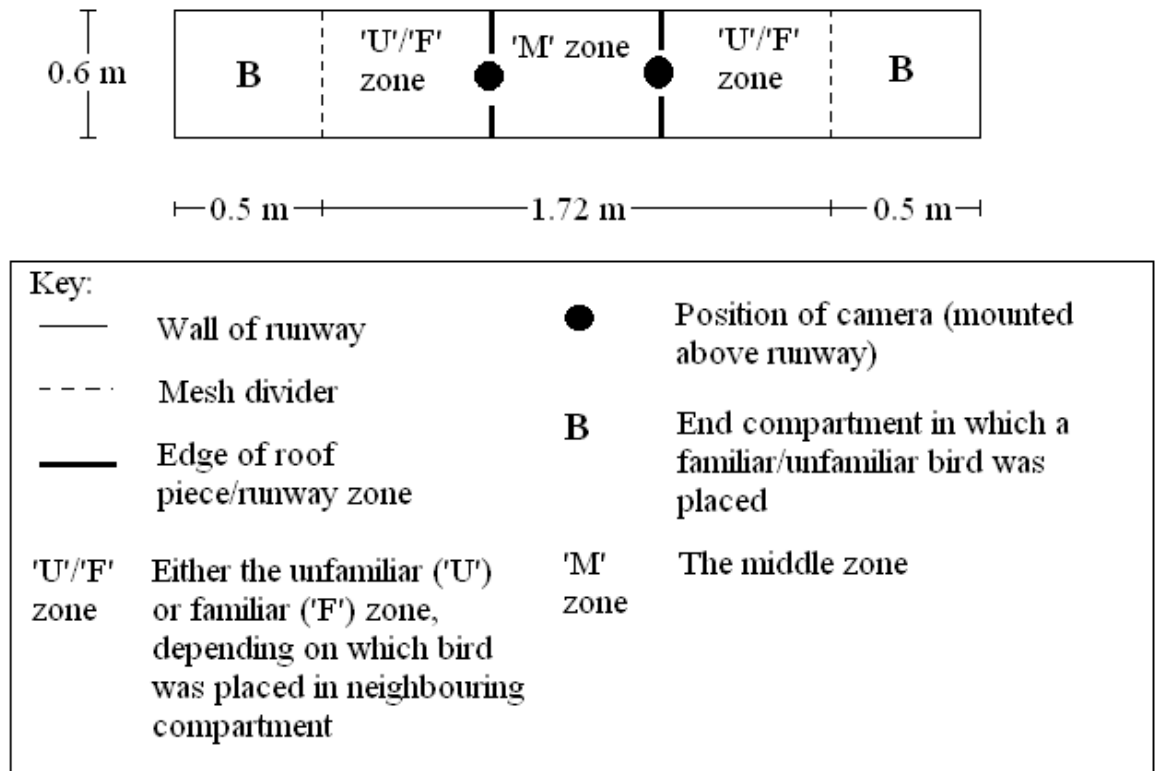


Figure 7 Diagram of runway apparatus used in Experiment 3 (not to scale)

3.2.4.2 Procedure

Each test bird was familiarised to the runway once a day over 3 days by placing each bird, individually, in the centre of the runway and leaving her in there for 30 s. Non-test birds were also familiarised to the runway, however, these hens were placed in one of the end compartments for 30 s.

All testing for this and following parts were conducted after 11:00 h to ensure hens were post-oviposition to avoid pre-laying behaviour. If a hen was showing signs of pre-laying behaviour, she was left for testing at 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 s prior to the test being placed in the middle zone. One of these non-test birds was the test hen's neighbour (i.e. a familiar bird), while the other was a pseudo-randomly chosen non-test bird which was chosen based on predetermined criteria (Table 12) so as to ensure that the hen was unfamiliar to the test hen and to minimise the possibility that the test hen had had visual contact with the hen. The ends at which the familiar and unfamiliar non-test hens were placed was randomly determined for each hen, each runway test. The runway zones were defined in accordance with the end in which the familiar and unfamiliar bird were placed; 'F' and 'U' respectively. The middle zone was defined as 'M'.

Table 12 Criteria for the choice of unfamiliar non-test birds in the runway test for Experiment 3

<p>Unfamiliar non-test hens were randomly chosen so long as:</p> <p>The hen was from a different tier of cages than that of the test hen,</p> <p>The hen was at least one pair of cages across (horizontally) from that of the test hen,</p> <p>The 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),</p> <p>The hen had not previously been utilised as an unfamiliar non-test hen for the test hen</p>
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In a randomly determined order, each test hen was placed in the runway in the ‘M’ zone and left in the runway for 3 min. The side from which she was placed in the runway was randomly determined. Runway tests were conducted three times a week (Monday, Wednesday and Friday) over 2 weeks for a total of six runway tests per bird.

Data collated from digital video recordings were latency to leave the centre zone (s), first zone entered and duration spent in each of the three zones (s). Due to technical problems the first day of runway tests were not recorded, therefore 5 days of runway tests were recorded and data collated.

Using results from the runway test, a familiarity score was calculated for each test hen by subtracting the proportion of time the hen spent in the ‘U’ zone from the proportion of time the hen spent in the ‘F’ zone, using data from all 5 tests.

3.2.5 Preference testing

3.2.5.1 Y maze apparatus

The Y-maze was a purpose built apparatus. The floor and removable roof pieces were constructed out of mesh, while the walls in the junction of arms (called the “choice area”) and arm gates were constructed out of solid metal (refer Figure 8). The start box had mesh walls and the gate between the start box and choice area was also mesh, 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 wall and roof piece mesh was a smaller square design.

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.

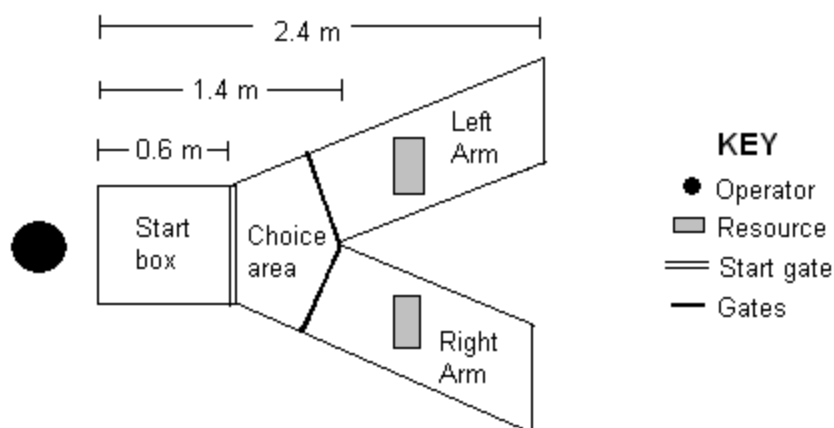


Figure 8 Diagram of Y maze apparatus (not to scale).

3.2.5.2 Familiarisation

Test hens were familiarised to the Y-maze twice over 4 days (alternate days) prior to training. This was deemed appropriate as the hens had already had experience being handled and being placed in a similar novel environment (i.e. the runway apparatus). In a randomly determined order, each hen was placed in the start box (SB) of the Y-maze in which both arm gates were open. After 10 s the SB gate was opened and the hen was allowed to leave. If the hen had not left within 30 s she was gently pushed forward. The SB gate was closed once she had left the SB. The hen was then given a further 30 s to move into one of the Y-maze arms. If she had not left the choice area, she was gently pushed forward towards the centre (where the two arms meet). Each hen was given a total of 3 min in the Y-maze once she had left the SB.

3.2.5.3 Training

Test hens were randomly allocated into one of three treatments ($n = 5$), which differed in the time allowed access to the dustbath in the Y-maze. The treatments were:

- “Short” = 2 min,
- “Intermediate” = 20 min,
- “Long” = 45 min

These times were based around the average duration of a dustbathing bout in laying hens of 27 min (Vestergaard, 1982). The quantity of time for social contact in the Y-maze remained constant at 5 min for all hens.

Training and testing were conducted on alternate days to coincide with the average occurrence of dustbathing behaviour (Vestergaard, 1982). Hens were socially isolated in their home cage by placing rubber partitions between pairs of cages and dustbaths were removed during the entire training and testing period.

Each hen was randomly allocated a Y-maze arm side (left or right) in which the dustbath was always placed. The dustbath was identical to the dustbath the birds had in their home cage and was refilled with peat moss between hens after every training/testing trial. The other arm contained the social resource. This was the test hen’s neighbour, which was confined in a mesh-fronted box at the end of the Y-maze arm (Figure 8, previous). If the Y maze arm in which the social option was placed had previously contained the dustbath, the arm was brushed down as to ensure no peat moss remained in the arm.

All birds were trained individually, in a randomly determined order on alternate days giving five training trials per bird. On each training session each hen had two consecutive trials in which one Y-maze arm and thus one resource was allowed access to at a time. The arm allowed access to first was determined randomly for each bird each training day.

Each hen was placed in the SB for 10 s before the SB gate was opened. The hen was allowed 30 s to move out of the SB, if she had not, she was gently pushed forward (with a flat hand on the tail) into the choice area. This interaction was referred to as a ‘handler intervention’. The SB gate was closed after the hen had moved out of the SB. Only one arm gate was open at a time allowing access to only one arm and thus resource. The hen remained in the Y-maze for the designated time (i.e. 5 min for social contact or the period of the treatment group for dust).

3.2.5.3 Testing

The Y-maze testing procedure was identical 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 into one of the Y maze arms, the

arm gate of the arm not chosen was closed and the hen remained in the maze for the required period (i.e. the treatment group period if dust was chosen or 5 min if the hen chose the social contact arm).

Each Y maze test was digitally recorded by three cameras mounted above the Y maze. Direct observations were also recorded for the latency to leave the SB (s), time to choice (s), Y maze arm/resource choice, number of 'handler interventions', whether a dustbathing bout occurred and, if so, the latency (s) for the bird to start dustbathing. In addition, the duration of dustbathing bouts (s) was recorded and whether the dustbath was completed or 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. The definition of a dustbathing bout from Table 11 was used to determine when a hen commenced and completed dustbathing bouts.

Each test hen had one Y-maze test trial per day on alternate days. Each bird had eight test trials. During the 16 days of testing, all hens were digitally video recorded for 24 hours per day and their time budget was recorded (in an identical manner as during the pre-experimental observations).

3.2.6 Statistical analysis

Each treatment's choice behaviour was compared to what would be expected at chance level (i.e. 50:50) by a Chi-square test.

Choice behaviour and time to choice data was analysed by one-way analysis of variance after transformation due to non-normality.

The familiarity score and dustbathing data were analysed by developing a parsimonious logistic over-dispersed generalised linear model relating the proportion of times hen dustbathe (when dust was chosen) to the familiarity score and treatment. The model chosen had parallel linear relationships between the log odds of the proportion and the familiarity score, for the three treatments.

A parsimonious general linear model relating the logarithm of average time dustbathing plus 100 to the familiarity score and treatment was developed. The model chosen had a separate linear relationship between the logarithm of average time dustbathing plus 100 and the familiarity score.

3.3 Results

3.3.1 Choice behaviour.

Hens in all treatments chose dust significantly more often than would be expected at chance level (i.e. 50:50) ($P < 0.001$ for all treatments). There was a tendency ($P = 0.07$) for birds in the intermediate treatment to choose social contact more often compared to both the long and short treatments (Figure 9).

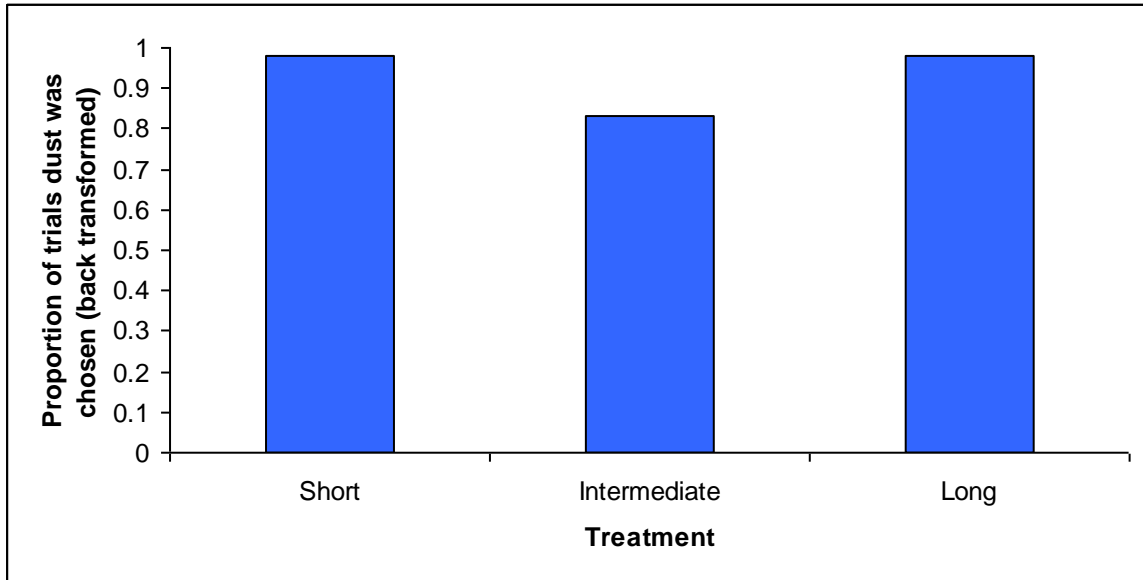


Figure 9 Choice behaviour (back transformed) for dust in Y-maze for Experiment 3.

3.3.2 Time to choice

Birds in the intermediate treatment were significantly slower to move through the Y-maze compared to the other treatments ($P < 0.001$) (Figure 5). There was no difference between the short and long treatments.

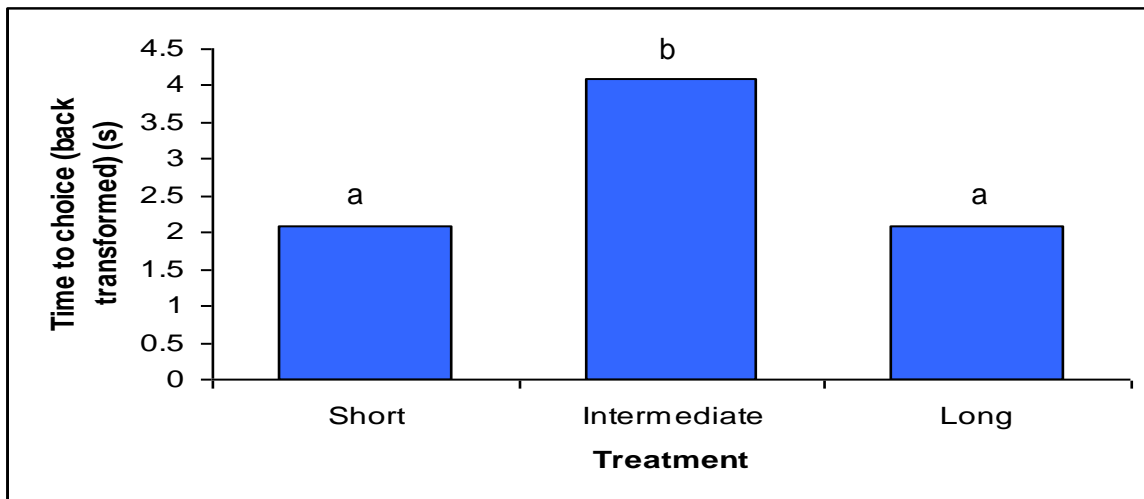


Figure 10 Time to make a choice (back transformed) in the Y-maze. Letters indicate significant differences ($P < 0.001$) for Experiment 3

3.3.3 Handler interventions

No birds required ‘handler interventions’ during preference testing.

3.3.4 Dustbathing

There was an effect of treatment on the proportion of dust chosen trials in which a dustbathing bout occurred. The long treatment had significantly higher proportion of dust-chosen trials in which there was a dustbathing bout compared to the short treatment ($P = 0.035$). The intermediate treatment did not significantly differ from either the long or short treatments.

When the relationship between the occurrence of dustbathing and familiarity score was examined, there was a significant treatment effect ($P = 0.035$) and familiarity score effect ($P = 0.014$) on the occurrence of dustbathing behaviour in the Y-maze (Figure 11).

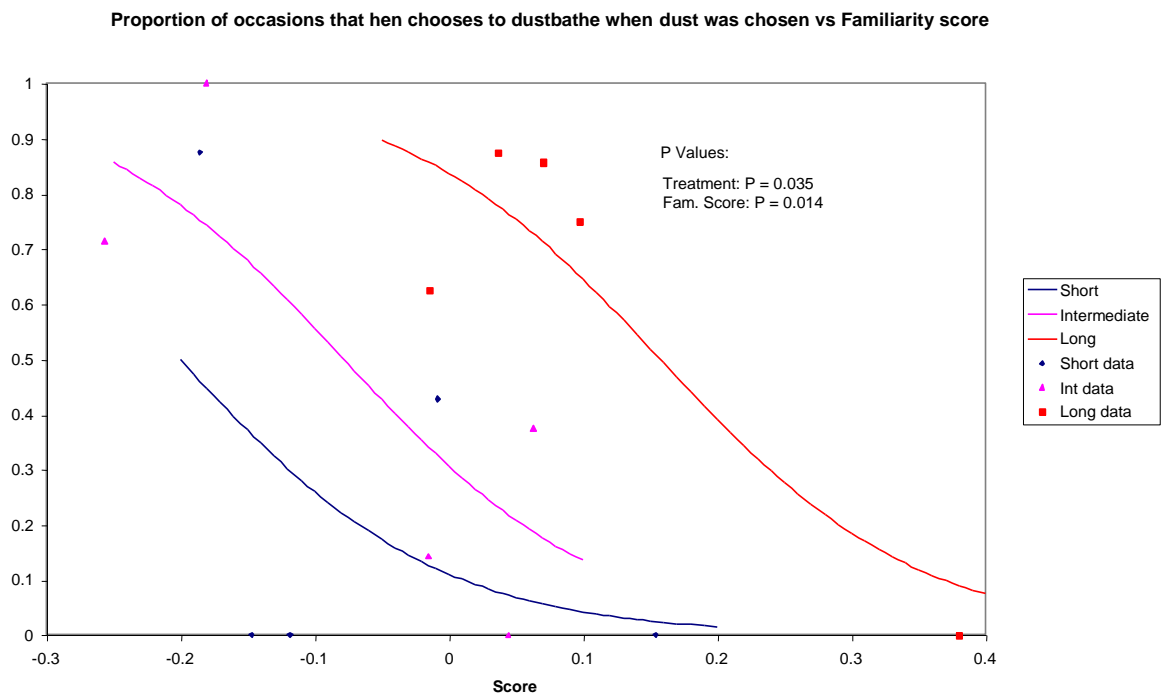


Figure 11 Proportion of dust-chosen trials in which a dustbathing bout occurred versus familiarity score in Experiment 3.

When the relationship between the duration of dustbathing in the Y-maze and the familiarity score was examined, there was a significant negative relationship the familiarity score and the duration of dustbathing in the Y-maze for both the long ($P = 0.002$) and intermediate ($P = 0.006$) treatments. The short group did not have this relationship ($P = 0.620$) (Figure 12).

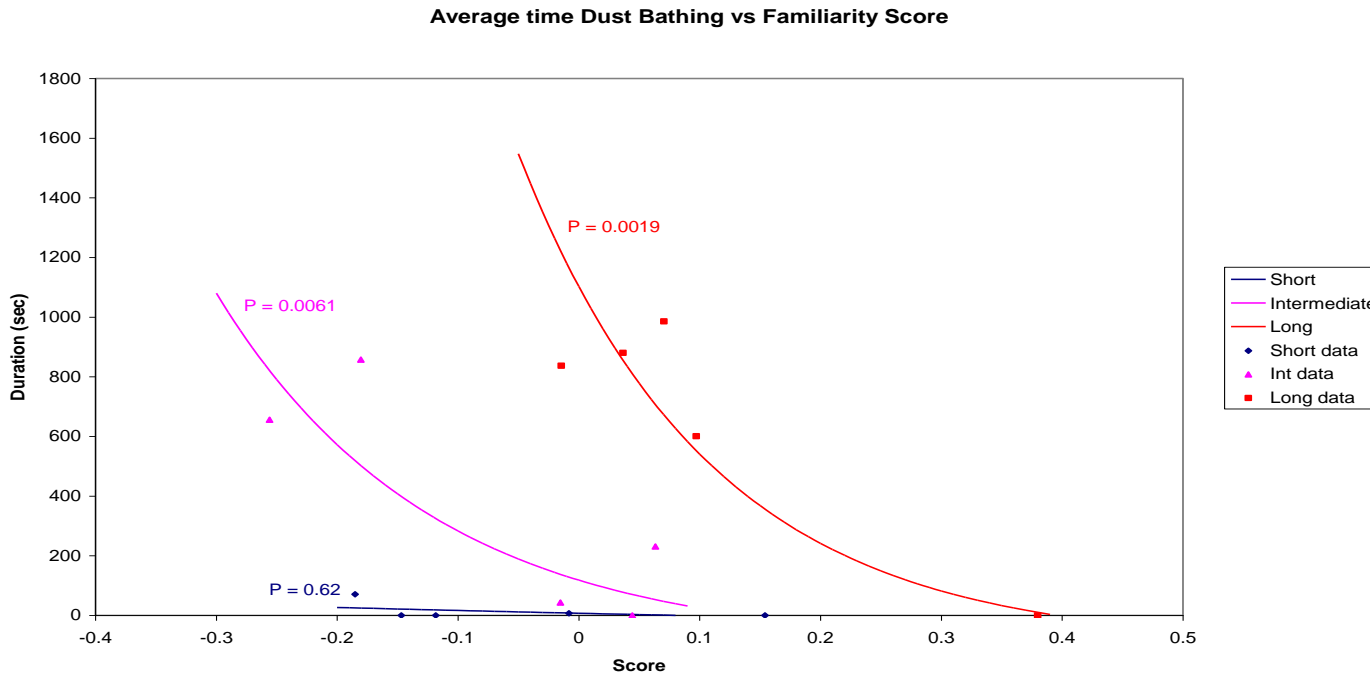


Figure 12 Duration of dustbathing bouts in the Y-maze versus familiarity score in Experiment 3.

3.3.5 Individual variation of use of dust substrate

When hens chose dust in the Y-maze a number of hens (n = 5) did not dustbathe in the dust on any of the trials that they chose dust (Figure 13). When hens did not dustbathe, they foraged with the dust. Conversely, a number of hens chose to dustbathe on every trial they chose dust.

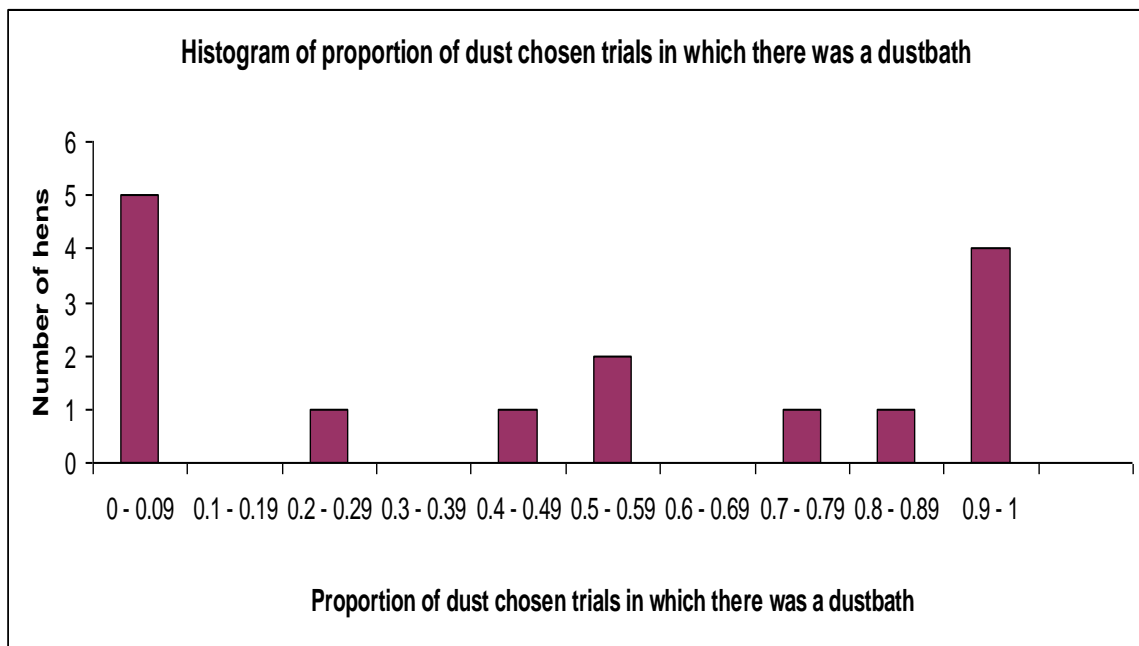


Figure 13 Histogram of the proportion of dust chosen trials in which there was a dustbathing bout

3.4 Discussion

The dust substrate provided in the Y-maze test proved to be an attractive resource to the test hens, as all treatments chose dust significantly more often than what would be expected at chance level. Even though the preference for dust was high, the intermediate group had a tendency to choose dust less often compared with the other treatments. The preference test trials were conducted every second day during the late morning to early/mid afternoon. This interval between test trials was chosen to coincide with the peak occurrence of dustbathing activity in unrestricted laying hens (Vestergaard, 1982) and therefore, presumed peak dustbathing motivation. Previous studies have shown that peat moss is the favoured dustbathing substrate of laying hens (Petherick and Duncan, 1989; de Jong *et al.*, 2007). Additionally, Wichman and Keeling (2008) found that hens, even without any experience of peat moss, are highly motivated to work for access to peat. Therefore, it may not seem unexpected that hens overwhelmingly chose dust as a dustbathing substrate. However, not all hens chose to dustbathe with the dust on every dust-chosen trial. Interestingly, there seemed to be some variation between individual hens in their utilisation of the peat moss presented in the Y-maze. Some hens dustbathed on every single trial they chose dust while conversely, some hens did not dustbathe at all when they chose dust (Figure 13). This type of individual variation in dust use was also observed in the study by Petherick *et al.* (1990). In the present study, birds that strictly ‘foraged’ or ‘dustbathed’ were observed in all treatments (refer Figure 11 and 12) and therefore this individual variation seems independent of time allowed with dust. Hence, the dust substrate in this experiment proved to be an attractive resource for both dustbathing and foraging activities.

The priority of access to a resource method utilised as the selection process for dominant and subordinate hens from the original group housing has had some criticism in the past (e.g. Rushen, 1983). This method was chosen in this study as a quick and efficient method to determine the relative social status of some of the birds. Studies that have found this technique invalid have tended to use a relatively short feed deprivation period and thus induce a relatively low motivation to feed. For example, Craig and Ramos (1986) used a deprivation period of only four hours. In contrast, the study by Banks *et al.* (1979) used a feed deprivation period of 18 hours and found that the priority of feed access was correlated with the social rank of the birds they tested (when they were observed for aggressive and submissive behaviours). Therefore, by incorporating a relatively long period of feed deprivation in this study and only choosing some of the hens, it is most probable that the hens were accurately chosen as either dominant or subordinate.

Chickens are highly social animals, however, although being socially isolated during the training and testing periods, hens chose social contact on only few occasions. The social deprivation carried out in the study prevented only visual and tactile contact with other conspecifics. Hens still had audible and olfactory contact with other birds. Also, while in the start box of the Y-maze, hens had visual contact with both resources in the Y-maze arms, which included another hen as the social contact option, albeit only briefly. Perhaps this short period of visual contact with another hen in the Y-maze, coupled with the sounds and smells of other hens while in the home cage was sufficient social contact for the test hens.

As well as having a tendency to choose dust less often, the intermediate birds were also slower to move through the Y-maze to make their choice (Figure 10). The speed of movement through the Y-maze and other tests where an animal must move to a resource can be equated to motivation (e.g. Petherick *et al.*, 1992). It would be expected that an animal with high motivation for access to a resource would move faster than an animal with low motivation. This may suggest that birds in the intermediate treatment had reduced motivation to gain access to a resource. Mason *et al.* (1998) suggested that repeated interruption of an activity might devalue the resource. Intermediate hens were allowed 20 minutes of dust access when they chose dust in the Y-maze. This period of time is less than that of the mean time of a laying hen dustbathing bout in an unrestricted situation (27 minutes, Vestergaard, 1982). Consequently, when an intermediate hen chose to dustbathe in a preference test trial, it is unlikely she would be able to complete the dustbathing bout and would be interrupted prior

to completion (i.e. picked up and moved back to her home cage by the experimenter). Perhaps the repeated interruption of dustbathing bouts devalued the dust resource for intermediate birds. This devaluation may have led to reduced motivation (and thus slower to move through the Y-maze) and also may have impacted on their choice for dust by having a tendency to choose dust less. Interestingly birds in the short treatment, which also would have had dustbathing bouts interrupted, did not show these indicators of dust devaluation as the speed of movement and choice behaviour was not different from birds in the long treatment. Perhaps the interruption of a dustbathing bout at the very beginning of a bout (i.e. within 2 minutes) is not as aversive to the dustbathing hen in comparison to a dustbathing bout interruption further in (as in the case of intermediate hens). This could explain the differences in motivation (i.e. speed of movement) and choice between these two groups.

The occurrence of dustbathing behaviour (when dust was chosen) in the Y-maze was essentially proportional to the length of time birds were allowed access to it. That is, birds in the long group had the highest occurrence of dustbathing, while the short group had the lowest occurrence of dustbathing. The two minutes allowed in the short group was probably not adequate for the duration of pre-dustbathing behaviour for most birds, although two out of the five birds did dustbathe on at least one occasion within the two-minute period. Even though there was less dustbathing, this did not impact on the choice for dust for the short treatment group, with the choice for dust identical to that of the long group. The long group had sufficient time to complete a full dustbathing bout. Even though this group had the highest incidence of dustbathing, one bird in this group did not dustbathe at all, even though she chose dust on every trial.

Even though all hens were familiarised to the runway apparatus, the runway tests may still have evoked fear in the hens as they were taken away from their home cage and placed in a novel situation. The presence of a conspecific in a novel environment can aid in the reduction of fear in chickens (Jones and Merry, 1988). 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 may suggest that in a fear-provoking situation, like that of the runway apparatus, birds choose to be closer to a familiar bird rather than an unfamiliar bird if they are fearful. This could imply that hens with a higher (or more positive) social 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.

An unfamiliar or novel environment, like that in the Y-maze apparatus, may potentially inhibit or reduce the quantity of dustbathing bouts compared to that observed in a familiar environment. Olsson and Keeling (2005) suggest that a bird is unlikely to commence a dustbathing bout unless the bird feels secure, as in the wild a bird is vulnerable to predators while dustbathing. If we presume that the hens which chose to spend more time near a familiar bird in the runway test (and therefore had a higher social familiarity score) were birds which were more fearful in the novel situation, this may help explain why these birds also dustbathed less in the Y-maze (Figure 11). This relationship between fear and dustbathing has also been observed in Japanese quail. Gerkin *et al.*, (1988) found that quail from a genetic line which were selected for high dustbathing, also displayed less when compared to the fear displayed by quail from low dustbathing line. Similarly, Marin *et al.* (2001) found sociality (as measured in a runway apparatus) was positively associated with stress. 'Stressed' chicks (chicks 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 the stimulus bird in comparison to 'non-stressed' controls. These results were more pronounced when a familiar chick was used compared to an unfamiliar.

The results from this preliminary study show that the quantity of reward has the potential to affect the choice behaviour of hens for peat moss and social contact and also that there are possible effects of preference for familiar birds on this choice behaviour. Reward size also had an influence on the time taken to move through the Y-maze, a possible measure of motivation. In addition, differing reward quantities seemed to affect the dustbathing behaviour in preference test trials. However the level of dustbathing activity may well be influenced by individual variation. Overall, results obtained warrant further, more detailed investigation to validate findings.

Experiment 4: Poultry surgical stress methods

4.1 Introduction

Intensively farmed animals are faced with an array of environmental stressors due to housing conditions and associated social interactions. In the past, both behavioural and physiological assessment techniques have been employed to assess the degree of stress the animal is under, but the appropriateness and accuracy of the physiological measures used has evoked conjecture in the scientific literature. In particular, the measurement of corticosteroids such as cortisol and corticosterone as an accurate indicator of the stress response has received significant attention. It is well established that when an animal is stressed the resultant release of the stress hormone corticosteroids (cortisol in many mammals or corticosterone in rodents and poultry) can be measured, and used as an indicator of such stress (Barnett, 2003; Mellor et al., 2000). The timing and frequency of sampling is very important in gaining accurate results, and the sampling techniques used can confound this if they in themselves are stressful. A previous study (Stevens, unpublished data) utilised a catheterisation technique to examine its efficacy in remote blood sampling. While the birds recovered well from surgery and showed no behavioural signs of distress either post surgery or during blood sampling, there was a drop in egg production by the birds. This drop in production suggests that the birds are experiencing greater stress as a result of the surgical implantation of the catheter than was anticipated. In order to ensure the usefulness of this catheterisation technique to allow us to measure baseline samples of corticosterone, we seek to examine whether this drop in egg production is related to a rise in the levels of circulating corticosterone. This experiment aims to examine the combined effects of surgery and repeated blood sampling on the behaviour, productivity and plasma corticosterone of the birds.

4.2 Methodology

4.2.1 Animals and Housing

All laying hens (Hy-line brown) were raised in group facilities located at Department of Primary Industries Victoria (DPI) research farm at Attwood in Victoria, Australia and relocated to the experimental facilities at 16 weeks of age, prior to onset of lay. The experimental facilities including home cages and y-maze testing apparatus were housed in a temperature controlled shed located at DPI Werribee, Victoria. Surgery was conducted in an adjacent room of this same shed. During the experiment birds were housed individually in cages (57cm x 50cm x 48cm). The birds had previously been exposed utilised in another experiment which utilised the y-maze and were habituated to frequent handling.

4.2.2 Experimental design

A total of 24 birds were utilised in this experiment with 8 birds in each of the 3 blood sampling treatments. The three treatments were utilised to look at the effect of repeated frequent blood sampling and also the effect of surgery on the birds. Treatment 1 birds did not undergo surgery, but were blood sampled from the wing vein 3 times, at 9am, 12pm and 3pm. Treatment 2 and treatment 3 birds underwent surgery to insert a catheter in the jugular vein. After recovery, treatment 2 birds were blood sampled 3 times on the one day at 9am, 12pm and 3pm, while treatment 3 birds were blood sampled every hour from 8am to 5pm on the same day.

4.2.3 Catheterisation

Birds were removed from food, the night before surgery but had free access to water. On the day of surgery the birds were removed from their home cage weighed and carried the surgical area adjoining their home shed. Prior to anaesthetisation with 1.5L/min oxygen and 0.5-1% isoflurane each bird received an intra muscular (im) injection of 0.1ml Atropine (), 0.5ml xylazine(). Once anaesthetised the birds were moved to lie on their left side and prepared for surgery. A round support was placed under their neck and the right hand jugular vein exposed, the feathers covering this area were held out of the way using clips. The area was disinfected and a small (~2cm) vertical incision made in the skin halfway down the neck near the right hand jugular vein. The jugular vein was dissected away from the surrounding fat and separated from the nerve running parallel to the vein. The vein was isolated with suture above and below the incision point prior to a small incision being made in the vein and the catheter inserted 9cm in the vein towards the heart. The catheter consisted of 2 pieces of tubing joined with a metal joiner, the section inserted in the vein was a 30cm length of size 3.0 CBAS™ heparin-coated polyurethane catheter tubing (Instech Solomon) with 1 movable bead placed 9cm from one end to mark the insertion depth. The other end was joined to 30cm of 22G CoEx™ polyethylene/PVC catheter extension tubing ending in a 22G blunt needle (Instech, Solomon) joined to a SAFSITE™ valve (B.Braun).

Once patency had been established the catheter was secured into the vein and then in a loop under the skin caudally to the insertion point. From here using a trochar the catheter was tunnelled under the skin and externalised on the back of the bird between the wings. The wound was sutured shut and engemycin applied topically in the wound and via an i.m. injection of 0.2ml. A small backpack consisting of a plastic tube (2cm diameter x10cm long) with a stopper at one end was used to hold the catheter. The catheter entered the backpack through a hole in the side in contact with the bird. The backpack was held on by elastic straps passing under the wings.

4.2.4 Blood sampling

4.2.4.1 From the Catheter

Birds were removed from their home cage prior to blood sampling, and placed on an elevated surface with a mesh similar to their home cage flooring. The bird was then lightly restrained by resting hands lightly on the wings of the bird while the catheter was removed from the back pack, a blood sample collected into a 1ml syringe before the catheter flushed with heparinised saline (25,000 units heparin per litre of saline) and returned to the backpack. Once the back pack was securely stoppered the bird was returned to the home cage. Collection of a blood sample took under 2minutes.

Once collected into a 1ml syringe the blood sample was transferred to a 1.2ml Lithium heparin monovette (Sarsted Australia, Technology Park, SA) at the end of the sampling period all blood samples were centrifuged at 6000rpm for 3min and the plasma removed to a 2ml microtube and placed and frozen. Plain sterile saline was added to the monovette to resuspend the red blood cells. Once resuspended the red blood cell/saline mix was transferred to a sterile tube and kept at 39°C in a water bath. The resuspended red blood cells were returned to the bird after every 3rd blood sample was collected, prior to the line being flushed clean i.e. after the collection of the 3rd blood sample 2ml of resuspended red blood cells from the 1st and 2nd blood samples were slowly infused in the catheter, the line flushed clean, the catheter returned to the back pack and the bird returned to its cage.

4.2.4.2 From the wing vein

Birds were removed from their home cage and carried to a bench for sampling. They were placed on their side with their feet restrained and the wing extended from the body, the person restraining the bird held back the feathers to expose the femoral vein. The blood sampler collected a 1.2 ml sample using a 23G x 1inch long needle attached to a 1.2ml Lithium heparin monovette. Once the sample was collected pressure was applied to the vein, before the bird was returned to its home cage being careful to maintain pressure on the wings and not allow flapping in order to reduce the incidence of

hematoma. Time taken to collect the sample was recorded. At the end of the sampling period all blood samples were centrifuged at 6000rpm for 3min and the plasma removed to a 2ml microtube and placed and frozen.

4.2.5 Behavioural observations

Video records were collected for a 24 hour period prior to surgery, through until 24 hours after blood sampling was completed. Time budgets of behaviour will be determined from these records.

4.2.6. Assays

Corticosterone will be determined using a commercial high sensitivity RIA kit.

An additional blood sample was collected at 9am from all birds and placed in an EDTA monovette for determination of white blood cell populations.

4.3 Results & Discussion

The behaviour and physiology data are presently being analysed and will be reported when completed to the Poultry CRC as an addendum to this report.

Experiment 5: The relationship between preferences and biological functioning of laying hens

5.1 Introduction

In an ethical analysis of an animal use, science can provide the factual basis of the impact of a husbandry or housing practice on the animal. However, there is considerable uncertainty within science on the concept of animal welfare. Scientists differ in their views on how animal welfare should be measured or judged, with three prominent concepts of animal welfare in the literature: the welfare of animals is judged on the basis of (1) how well the animal is performing from a biological functioning perspective; (2) affective states, such as suffering, pain and other feelings or emotions; and (3) the expression of normal or 'natural' behaviours. These different concepts or views on animal welfare lead to the different methodologies to assess an animal's welfare.

While there is a wide acceptance of the scientific method in problem solving, its ability to contribute to the welfare debate has been limited to some extent by a lack of consensus on the scientific approach to studying animal welfare. For many scientists, animal welfare is defined and measured on the basis of how well the animal is performing from a biological functioning perspective, while for others it includes animal preference on the basis that animal preferences are either influenced by the animal's emotions, which have evolved to motivate behaviour in order to avoid harm and facilitate survival, growth and reproduction, or reflect important biological requirements of the animal. Another concept in the literature is the one predicated on the view that the welfare of an animal is improved in environments or situations in which the animals display normal or 'natural' behaviour.

There are several commonalities in the rationale for these approaches. For example, it is considered that animals, at least in the wild, will be motivated to choose those resources or behaviours that maintain homeostasis or biological functioning to optimise their fitness, that is, optimise their growth, reproduction, injury status, health and survival. Furthermore, feelings or subjective affective states have evolved to motivate behaviour in order to meet needs that have to be satisfied in order for the organism to survive, grow and reproduce.

This conceptual convergence suggests a way forward in developing a broader consensus on the study of animal welfare by reducing both conceptual differences and consequently methodological differences in animal welfare science. The validity of the welfare criteria can be tested in several ways: first, with the finding that there are correlations between independent measures of different concepts of animal welfare and second, with the finding that intuitively aversive conditions reduce animal welfare on the basis of the measures of different concepts of animal welfare.

Therefore research examining the validity of these concepts, and in turn methodologies, is necessary to understand the relationships between the concepts and indeed minimise the conceptual and methodological differences as discussed above. This experiment, which is one in a series of experiments with pigs and poultry, will contribute to the validation of welfare research methodology and thus the value of the research is that it is necessary to develop well-accepted welfare methodology that can be used by animal welfare scientists to assess animal welfare requirements in a broadly agreed manner. This experiment examined the individual preferences of laying hens for dust-bathing and social contact and the effects of deprivation of the more preferred resource (dust-bath or social contact) on biological functioning in individual birds.

5.2 Methodology

5.2.1 Part 1

The choice behaviour of 48 40 week-old commercial laying hens (*Gallus gallus domesticus*), individually housed indoors in cages (57cm x 50cm x 48cm), for a dust-bathing substrate vs. social contact were studied in a series of Y-maze trials over 24 days. The preferences were studied firstly over 7 days when birds had continuous access in their home cages to both a dust-bathing substrate (sawdust) and social contact (visual and tactile contact with neighbour) and secondly over 7 days when deprived in their home cages of both social contact (visual and tactile contact) and a dust-bathing substrate.

During the study, the birds were fed a commercial layer ration (pelleted form) at a maintenance level. This was calculated using the equation:

$$ME(\text{kJ/day}) = W(586 + (-8.4T)) + 8.4E$$

Where ME = metabolisable energy (kJ/day), W = individual bird weight (g), T = temperature (Celsius) and E = egg output (g/day) (Chwalibog and Baldwin 1995).

Water was available ad libitum via a drinker at the rear of the cage. All birds had at least auditory and olfactory contact with other birds. The hens were kept in commercial conditions: a constant environmental temperature of 21°C and a light:dark cycle of 16:8 hours.

To study preferences for the two resources under study (social contact vs. dust-bathing substrate), the choice behaviour of the birds were examined in a series of 'competitive' Y-maze trials. The birds were initially trained to the Y-maze with one resource in each arm. Resources, either social contact, involving tactile and visual contact with familiar birds, or a dust-bathing substrate in a tray, were randomly allocated to arms for each bird, so that birds learnt to associate each arm with one resource.

Training, familiarisation and testing for the entire study began at 1030 h each day. This start time was considered the optimal time to avoid pre-laying behaviours associated with oviposition whilst still completing the 24 trials in one day. If a bird displayed pre-lay behaviours at the time of familiarisation, training or testing, that hen was run at the end of the scheduled session for that day.

5.2.1.1 Pre-experimental phase

48 birds were tested in two groups of 24 individuals. Birds in the first replicate were put into individual cages where dust, feed and social contact were readily available. During this time birds in the second replicate, still housed in group cages, were given access to dust topped up several time a day. Each replicate of birds was acclimatised to routine handling using the same methods outlined in Experiment 2 during the week following the move to individual cages.

5.2.1.2 Familiarisation

Familiarisation took place over 5 days and followed the same procedure outlined in Experiment 2

5.2.1.3 Training

The birds were trained to use the Y-maze complete with the two resources in the arms, via one "forced" entry per day to each arm over 5 days following a similar procedure to that outlined in Experiment 2.

On each day of the training period, birds were allowed 30 s to move out of the start-box before movement was gently encouraged by pushing the bird forward with a flat hand on the tail. Once out of the start box the birds were given 30 s to enter the designated maze arm before they were encouraged to move forward. Once in the arm the bird was given 5 minutes with the resource. The bird was then removed from the Y-maze and the process was repeated for the other arm. The order of exposure to each arm was randomly allocated. During the training phase, dust and social contact was removed/restricted 30 minutes prior to being put in the maze. On completion of each trial birds were returned to their home cages with social contact and dust available *ad libitum*.

5.2.1.4 Testing

In the following 14-day testing phase, choice behaviour was studied in daily trials, in which birds could access either resource via one arm. In each trial birds were introduced individually to the starting box. As with the training and familiarisation the birds were given 30 s to leave the start box and once out of the start box, the bird was allowed 30 s to move down one of the arms. On entry to an arm, the gate on the other arm was closed and the bird remained within the arm with the chosen resource for 5 minutes. As in the training phase, the birds will be given 30 s to leave the start box and 30 s to enter an arm before they are encouraged to move forward by gently and briefly pushing them as in previous experiments.

In each trial birds were allowed a free choice of arms (dust vs social contact (familiar bird)). During the 2 weeks of testing, choice behaviour was studied firstly over 7 days when birds in their home cages had continuous access to both a dust-bathing substrate and social contact (as in the training phase dust and social contact were restricted in the home cages 30 minutes prior to testing and were returned *ad libitum* upon completion of testing), and secondly over 7 days when deprived in their home cages of both social contact (visual and tactile contact) and a dust-bathing substrate. Each bird was tested daily over these 14 days, with one non-test day between introduction of *ad libitum* and restricted resources in the home cage to modify the cages. Thus a total of 25 days was used, 10 days of training in the Y-maze followed by 2 weeks of testing, with one day to set up cages.

5.2.2 Part 2

Upon completion of the Y-maze testing, each replicate was re-grouped into their original group cage arrangements for a minimum of 1 week, during which time the birds were regularly handled and had access to both dust and obviously social contact. Once the second replicate was completed, 24 birds were selected from Part 1 for Part 2 (see below) and housed individually in new cages with tactile and visual contact with neighbouring birds and with dustbaths for three weeks prior to commencement of Part 2.

From the 40 birds studied in the first part of the experiment, 24 were selected: 12 birds that chose social contact in the majority of Y maze trials (labelled “social preferred” group of birds) and 12 birds that chose dust in the majority of Y maze trials (labelled “dust preferred” group of birds). Birds from each of the two groups were housed in pairs, with each pair housed individually in adjacent cages (with or without a solid dividing wall) and with solid walls between adjacent pairs. Over a 6 week period, half of each of the two groups birds (ie. 6 social preferred and 6 dust preferred birds) was housed in one of the two following restrictions:

- Social restriction only – individually housed with restricted social contact through the elimination of both visual and tactile contact with birds but not olfactory and auditory contact with birds. The birds were provided with fine-grain sawdust in 46.6 x 22.6 x 3.2 cm plastic tray in the cage.
- Dust restriction only – the birds were not provided with a dustbath in their accommodation cage but had visual and tactile contact with the neighbouring bird (other member of the pair) through the wire-mesh side wall.

The experimental unit in the study is the pair of birds (birds in a pair will be from the same preference cohort and will undergo the same housing treatment) and birds in a pair will have no visual or tactile contact with other birds while in their home cages.

5.2.3 Behaviour

Cameras were mounted over the cages to record the behaviour of the birds on one day in each of weeks 1, 3 and 5 of the 6-week study period. From the digital video records, instantaneous sampling at 15-min intervals from 0700 to 1700 h was used to record whether or not each of the following postures and behaviours were shown by each bird on each observation day: standing (either idle, interaction with neighbour, dustbath, pen fittings, floor, feeder, drinker, elimination, etc) and crouching.

5.2.4 Live weight

Birds were weighed at the start and completion of Part 2.

5.2.5 Stress physiology

After 5 weeks of treatment, the birds were catheterised and 5 days later serial blood samples (1.2 mL) were collected via the catheter, using Monovettes (Sarstedt Australia, SA) coated with lithium heparin, at 1-h intervals between 0700 and 1700 h (red blood cells were returned to the bird every third bleed), to measure the day-time profile in corticosterone concentrations of the birds. Single intra-muscular injections of adrenocorticotrophic hormone (ACTH; 12.5 IU Synacthen, Ciba Geigy, Lane Cove, NSW) were administered to the birds at 0800 h at 6 and 8 days post-surgery, respectively, and blood samples were collected (as described above) immediately prior to each of the injections and then hourly for 9 h post-injection. The rationale for the 'ACTH' test is that chronic stress generally results in higher cortisol responses to exogenous ACTH (Dantzer and Mormède 1983; Meunier-Salaun et al. 1987; Barnett 1997). The blood samples were centrifuged and the plasma was stored frozen at -18°C until assayed for total (ACTH response and 'day-time profile') and free corticosterone ('day-time profile') concentrations.

An extra blood sample (1.2 mL) was collected with the first bleed on Day 5 post-surgery via the catheter using EDTA coated Monovettes (Sarstedt Australia, SA). This sample was used to measure neutrophil and lymphocyte (absolute and proportional) numbers and the ratio between neutrophil and lymphocytes.

5.3 Results & Discussion

The behaviour and physiology data are presently being analysed and will be reported in June 2009 to the Poultry CRC as an addendum to this report.

General Discussion

A current weakness in assessing animal welfare and subsequently in establishing welfare standards for animals is that there are differing definition of animal welfare, which provoke considerable debate on animal welfare assessment and standards. This unease with the definition exists both within science and more broadly when decisions on acceptable welfare standards are being made by individuals or the community.

While there are several concepts of animal welfare in the literature, scientists have basically used two methodologies to study animal welfare: the welfare of animals has been assessed on the basis of either biological functioning or animal preferences. The first approach is an integrated one measuring behavioural, physiological and health and fitness responses to assess biological functioning on the basis that difficult or inadequate adaptation will generate welfare problems for animals. The second uses animal preference (and behavioural demand) testing on the basis that animal preferences are influenced by the animal's emotions, which have evolved to motivate behaviour in order to avoid harm and facilitate survival, growth and reproduction.

This scientific uncertainty in relation to animal welfare concepts or views does not necessarily diminish the robustness of the research utilising criteria or methodologies promulgated by these different views or concepts. However, it does raise the question of the relatedness of these concepts. In other words, is biological dysfunction associated with or does it lead to negative affective states and vice versa? Thus, are the resultant methodologies measuring the same state(s) in the animal? Alternatively, if different methodologies measure different aspects of the animal's welfare, which aspects are most important for overall welfare? Research utilising well-accepted stress models is required to understand the relationships between these concepts and methodologies. Fundamental research, with the overall objective of integrating these criteria and developing a broader consensus on animal welfare methodologies, may assist in reducing the interpretative differences in animal welfare science, and this is the general aim of this project. To examine the relationships between two concepts of animal welfare, the biological functioning and feelings-based concepts, this project aimed to test the hypothesis that deprivation of a preferred resource results in biological dysfunction. A range of behavioural, physiological and fitness responses were used to assess biological functioning in this experiment, while bird preference was assessed by offering animals a choice of resources in a series of Y maze trials.

Since preference testing methodologies are less well established than those utilized to assess biological functioning, a significant component of this project was committed to developing a valid and easily applied methodology to assess choice behaviour in poultry. A valid methodology was considered on the basis of (1) the consistency of choice behaviour, both within and between animals, and (2) animals consistently chose the expected desirable resource.

In the first two experiments, Y maze tests were used to examine preferences for feed, social contact and a dustbathing substrate (sawdust). In the first experiment, the hens' choice for feed was higher than that expected if the choice was random for both those deprived of feed for 30 min or 4 h prior to testing. In contrast, the hens' choice for dustbathing substrate was not different from that expected if the choice was random for both groups of hens. These results are similar to Petherick *et al.* (1993) who also found hens chose feed significantly more often compared to a dustbathing substrate regardless of deprivation treatments, suggesting a high attractive value for feed. From an evolutionary perspective, food is a very important resource as it is a necessity for animal survival. Therefore, it is not surprising that hens chose this resource on the majority of trials. The findings from this first experiment indicate that laying hens are able to make feed choices in a Y-maze. In the second experiment, hens consistently chose feed over dust or social contact, irrespective of whether the birds were deprived of the resources before testing (3 h for feed and 12 h for dust or visual contact with birds). The proportion of choices for feed was significantly greater than that for a random choice pattern (i.e. 50%). In contrast, the overall choice for dust and social contact was similar (46 vs. 54% for dust and social

contact, respectively). Thus these results indicate a consistent and strong relative preference for feed over dust and social contact in laying hens.

It is important to note that the levels of restriction studied in these two experiments were arbitrary, with the utility (i.e. reward value) of the resource for restricted birds likely to differ between resources, and thus any interpretation of the relative attractiveness of the resources based on these arbitrary restrictions must be made cautiously. It is clear from the results of the two experiments that birds consistently chose feed over dust or social contact, irrespective of restriction of any of these resources. This result that the preference for a biological necessity such as food overrides that of social or dustbathing preferences, makes evolutionary sense and is in agreement with other preference methodologies which have indicated the value of a feed resource exceeds those of other resources examined, and is often assumed to demonstrate the maximal response or preference in most cases (e.g. Dawkins, 1983).

It appears that these design features of preference tests continue to be arbitrary, as authors often fail to describe why or how certain aspects of their design were chosen. Indeed, design features such as the number of test trials, the frequency of testing, the quantity of reward and the quality of reward may affect choice behaviour and thus lead to spurious results. Thus the third experiment in this project examined the importance of one aspect of the design of the preference test, quantity of the reward. The quantity of reward in a Y-maze preference test refers to the quantity of time the animal is allowed contact with its chosen resource. This value may potentially affect the choice behaviour of an animal in a preference test by making the resource more or less attractive. This experiment examined the effects of quantity of reward in a Y-maze preference test on laying hens. The resources in the Y-maze were dust (a tray of peat moss) or social contact (the presence of a familiar subordinate hen) and hens were allocated into one of three treatments, which differed in the quantity of time allowed with the dust resource when it was chosen; 'short' (2 minutes), 'intermediate' (20 minutes), 'long' (45 minutes). The quantity of reward for social contact remained 5 minutes for all birds. During testing, hens were deprived of both dust and social contact in their home cage. All hens chose dust significantly more than chance level, however, the intermediate treatment had a tendency to choose social contact more often than the other treatments. In addition, the intermediate treatment was slower to move through the Y-maze compared to the other treatments. Overall, these results indicated that while there was no significant effect of the quantity of reward on choice behaviour, birds which received the intermediate reward quantity, when compared to the other treatments, showed evidence of reduced motivation to obtain dust as demonstrated by their tendency to choose social contact more often and their reduced speed of movement. The results of this preliminary study show that the quantity of reward has the potential to affect the choice behaviour of hens for peat moss and social contact and also that there are possible effects of preference for familiar birds on this choice behaviour.

These results also highlight the need to understand the effects of design features of preference tests (e.g. time with resource, social context, resource deprivation level, etc.) on the choice behaviour of laying hens in Y-maze preference tests. This knowledge is necessary if the measurement of the choice behaviour is to be used to answer questions about animal welfare. Further studies in a PhD program at the University of Melbourne are continuing to study the effects of design features of preference tests on the choice behaviour of laying hens in Y-maze preference tests.

The experiments discussed so far have developed the preference testing methodologies and advanced our understanding of resource deprivation on hen preferences. The fourth experiment in this project investigated the biological functioning of laying hens in response to a significant stressor, that of surgery and repeated blood sampling. Biological functioning was measured using behaviour, productivity and plasma corticosterone variables. The behaviour and physiology data are presently being analysed and will be reported in June 2009 to the Poultry CRC as an addendum to this report.

The ultimate aim of this project was to study, using well-accepted stress models, the relationships between two concepts of animal welfare, the biological functioning and feelings-based concepts. One way to study these relationships is to test the hypothesis that deprivation of a preferred resource results in biological dysfunction and Experiment 5 in the project examined the effects of deprivation of a dust-bath or social contact of birds that either preferred for dust-bathing and social contact on

biological functioning. From the 40 birds studied, 24 were selected: 12 birds that chose social contact in the majority of Y maze trials (labelled “social preferred” group of birds) and 12 birds that chose dust in the majority of Y maze trials (labelled “dust preferred” group of birds). Over a 6 week period, half of each of the two groups birds (ie. 6 social preferred and 6 dust preferred birds) was housed in one of the two following restrictions:

- Social restriction only – individually housed with restricted social contact through the elimination of both visual and tactile contact with birds but not olfactory and auditory contact with birds. The birds were provided with fine-grain sawdust in 46.6 x 22.6 x 3.2 cm plastic tray in the cage.
- Dust restriction only – the birds were not provided with a dustbath in their accommodation cage but had visual and tactile contact with the neighbouring bird (other member of the pair) through the wire-mesh side wall.

Over this 6-week period, measurements were conducted on the behaviour of the birds in terms of postures and behaviours, live weight and stress physiology. The behaviour and physiology data are presently being analysed and will be reported in June 2009 to the Poultry CRC as an addendum to this report. It is of interest to briefly report on a related but more-advanced project on pigs that may contribute to the validation of welfare research methodology.

One experiment in a series of experiments in this related pig program has recently been completed. The main aim of this experiment (Hemsworth et al., unpublished data) was to examine the relationship between two methodologies to assess animal welfare, biological functioning and animal preferences, and the specific hypothesis tested in this experiment was that deprivation of the pig’s more preferred resource, feed or social contact, results in biological dysfunction. Two interesting interactions were found that provide limited support for the hypothesis that deprivation of the pig’s more preferred resource, feed or social contact, results in biological dysfunction. There was a significant interaction between the main effects of deprivation and preference on live weight: feed preferred pigs weighed less than social preferred pigs when both groups were deprived of feed, while socially preferred pigs weighed less than feed preferred pigs when both groups were socially deprived. There was also a tendency for an interaction between main effects on free cortisol concentrations: when deprived of feed, the feed preferred pigs tended to have higher cortisol concentrations than social preferred pigs while the socially preferred pigs tended to have higher cortisol concentrations than the feed preferred pigs when deprived of social contact. In another experiment in this series (Hemsworth et al., unpublished data) in which 75% of the study pigs preferred social contact over feed, social deprivation reduced growth rate of the study pigs. While there was no evidence that social deprivation suppressed feed intake, the authors concluded that a likely interpretation of the reduced average daily growth in the socially-deprived pigs was the catabolic effects of ACTH and corticosteroids (Elsasser et al., 2000): social deprivation, through the energetic cost of coping with a stressful situation, may have reduced average daily growth.

These results on pigs provide limited evidence that deprivation of a highly preferred resource may result in biological dysfunction on the basis of a changes in free cortisol concentrations and live weight. While 48 pigs were studied in the second experiment, only 16 pigs were studied in the second experiment and the interaction on free cortisol concentration was a tendency only. Clearly, further research is warranted to address this effect. Experiment 5 in this Poultry CRC project will contribute to our understanding of the effects of deprivation of highly preferred resources on biological functioning and thus provide an insight into the relationship between these two methodologies of animal welfare assessment. Preference methodologies may provide a reliable measure of animal choices. However, with our present limited knowledge of the relationship between these two methodologies, in order to be confident that the outcomes of preference tests reflect the welfare requirements of the animal, additional evidence, particularly on the occurrence of abnormal behaviour, stress physiology and health when restricted of the resource or the behaviour of interest, would be prudent to provide a more comprehensive assessment of the impact of restricting a resource or behaviour on animal welfare (Widowski and Hemsworth, 2007). The development of a broader scientific consensus on welfare measures arising from this research should lead to the development of more credible measures that can be used independently or incorporated into welfare assessment (and screening tools in the field).

Implications

It is not entirely clear how individuals make decisions about the acceptability of specific animal uses. In an ethical discussion of a particular animal use, a range of considerations may affect this decision. An individual's attitude to the animal in question as well as an individual's attitudes to society's obligations to animals together with what science may tell us about the impacts of the use on the animal are all likely to be influential. Furthermore, broader risks and harms, such as environmental, economic and social considerations, may also be utilized. Philosophical views and religious and cultural beliefs about a particular animal use may also affect the individual's decision on this animal use. Nevertheless, it is clear that as society continues to ask questions about appropriate welfare standards for domestic animals in a range of farming, domestic, recreational and research activities, there will be an on-going requirement for science to provide the factual basis for an ethical analysis of the impact of society's use of animals on the animal itself.

In an ethical analysis of an animal use, science will provide the factual basis of the impact of a husbandry or housing practice on the animal, in particular its impact on the welfare of the animal. Evidence of animal suffering, in terms of prolonged adverse physiological and mental states in an animal, is an important consideration in rejecting a particular animal use. However, there is considerable uncertainty within science on the concept of animal welfare. Scientists differ in their views on how animal welfare should be measured or judged, with three prominent concepts of animal welfare in the literature: the welfare of animals is judged on the basis of (1) how well the animal is performing from a biological functioning perspective; (2) affective states, such as suffering, pain and other feelings or emotions; and (3) the expression of normal or 'natural' behaviours. These different concepts or views on animal welfare lead to the different methodologies to assess an animal's welfare.

This 'conceptual' uncertainty is one of the most obvious and challenging limitations for science in relation to its contribution to establishing and verifying animal welfare standards. This conceptual uncertainty has several implications for identifying and resolving genuine risks to an animal's welfare. First, while there are several concepts of animal welfare in the literature, scientists have basically used two methodologies to study animal welfare: the welfare of animals has been assessed on the basis of either biological functioning or animal preferences. The first approach is an integrated one measuring behavioural, physiological and health and fitness responses to assess biological functioning on the basis that difficult or inadequate adaptation will generate welfare problems for animals. The second uses animal preference (and behavioural demand) testing on the basis that animal preferences are either influenced by the animal's emotions, which have evolved to motivate behaviour in order to avoid harm and facilitate survival, growth and reproduction, or reflect important biological requirements of the animal. Therefore, differences in concepts and thus definitions of animal welfare within science lead to differences in the methodology used by scientists to assess animal welfare under different husbandry or housing practices.

Second, differences between policy makers on the concept and definition of animal welfare can lead to disagreement on animal welfare-related policy and legislation. While decisions on specific animal use are affected by a number of considerations including scientific information of the harms and benefits to the animal, differences in concepts, definitions and, in turn, assessment lead to differences between policy makers in industry, community groups and Government in their interpretation of the validity of scientific information arising from a specific methodology. Consequently, these differences between policy makers in interpreting similar information can lead to disagreement on setting or accepting specific animal welfare standards. For example, an increasingly predominant view, that has the support of many welfare groups worldwide, often places more weight on the importance of 'natural' animal behaviour in more natural settings than the normal biological functioning of the animal in assessing animal welfare. This arguably uncritical and subjective approach is likely to have a major impact on intensive animal use and paradoxically may not improve animal welfare.

Third, it is important in any welfare monitoring scheme in the field that the emphasis should be on the animal itself and thus on those measures that best reflect a lack of animal suffering. The welfare

measures or 'tools' that science develops to evaluate the welfare implications of husbandry and housing practices will obviously be incorporated into welfare assessment and screening tools in the field. Credible field measures are critical in providing assurance to the industry, markets and regulatory authorities. Thus any uncertainty about the validity of the scientific measures on which the field measures are based will affect community, consumer, industry, community group and Government confidence in compliance with specific welfare standards.

This scientific uncertainty in relation to animal welfare concepts or views does not necessarily diminish the robustness of the research utilising criteria or methodologies promulgated by these different views or concepts. However, it does raise the question of the relatedness of these concepts. In other words, is biological dysfunction associated with or does it lead to negative affective states and vice versa? Thus, are the resultant methodologies measuring the same state(s) in the animal? Alternatively, if different methodologies measure different aspects of the animal's welfare, which aspects are most important for overall welfare? The research conducted in this Poultry CRC project will contribute to a better understanding of the relationships between these concepts and methodologies. While the analyses in the final experiment in this project are presently incomplete, it is clear that further research is necessary to comprehensively test the underlying hypothesis in this research program. The outcome of this fundamental research endeavour is the integration of these welfare criteria and the development of a broader consensus on animal welfare methodologies to assist in reducing the interpretative differences in animal welfare science.

Recommendations

The present research, together with previous research, show that preference methodologies may provide a reliable measure of animal choices. However, as with biological dysfunction, clarifying the conceptual link between animal preferences and animal welfare is an issue for many. The individual's concept of animal welfare clearly underscores the methodology used to judge or measure animal welfare. Testing this project's hypothesis will contribute to our understanding of the effects of deprivation of highly preferred resources on biological functioning and thus provide an insight into the relationship between these two methodologies of animal welfare assessment. The finding that deprivation of the resources most preferred by the animals results in biological dysfunction would lead to the development of a broader scientific consensus that either of these two methodologies can be used independently or together to judge animal welfare in research settings (and in screening tools in the field).

Until this hypothesis is comprehensively tested, with our present limited knowledge of the relationship between these two methodologies, in order to be confident that the outcomes of preference tests reflect the welfare requirements of the animal, additional evidence, particularly on the occurrence of abnormal behaviour, stress physiology and health when restricted of the resource or the behaviour of interest, would be prudent to provide a more comprehensive assessment of the impact of restricting a resource or behaviour on animal welfare (Widowski and Hemsworth, 2007).

The overall recommendation is that the research commenced in this Poultry CRC project continue to comprehensively test the hypothesis that deprivation of a preferred resource results in biological dysfunction. However, several additional recommendations are made that should be followed before this overall recommendation is acted on. The project's proposed approach in understanding the relationships between welfare concepts or methodologies is not the only approach in establishing more credible welfare methodologies. There is a number of international research projects that are using preference research integrated with other measures and such research along with that proposed here will assist in the development of a broader scientific consensus on welfare measures used in animal welfare research. Thus there needs to be a review of current research on welfare methodology, both in Australia and overseas, to examine the opportunities for coordination of this research. More active international collaboration where appropriate should be sought.

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Plain English Compendium Summary

As part of the Final Report requirements, authors need to provide a one page, plain English Compendium summary along with each Final Report in electronic and hard copy format. These compendium summaries are published in Poultry CRC’s annual Report. A template for the summary can be found below, following the completed example.

Note that this one-page summary will be read by people without expertise in the field of study. It should therefore be as easy to read and understand as possible

Plain English Compendium Summary

Project Title:	Improving the scientific assessment of poultry welfare
Poultry CRC Project No.:	04-18
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Project Overview	There are several concepts of animal welfare in the scientific literature, which have led to uncertainty in relation to both welfare methodology and interpretations. The specific objectives of this project were (1) to develop a suitable methodology for preference testing to measure the animal’s longer term choice for specific resources and (2) compare the two main approaches to assess welfare: “normal biological functioning” and “preference testing”.
Background	<p>While there are several concepts of animal welfare in the literature, scientists have basically used two methodologies to study animal welfare: the welfare of animals has been assessed on the basis of either biological functioning or animal preferences.</p> <p>This conceptual uncertainty has several implications for identifying and resolving genuine risks to an animal’s welfare. Differences between policy makers in interpreting similar information can lead to disagreement on setting or accepting specific animal welfare standards. For example, an increasingly predominant view, that has the support of many welfare groups worldwide, often places more weight on the importance of ‘natural’ animal behaviour in more natural settings than the normal biological functioning of the animal in assessing animal welfare. This arguably uncritical and subjective approach is likely to have a major impact on intensive animal use and paradoxically may not improve animal welfare.</p> <p>This scientific uncertainty in relation to animal welfare concepts or views does not necessarily diminish the robustness of the research utilising criteria or methodologies promulgated by these different views or concepts. However, it does raise the question of the relatedness of these concepts. In other words, is biological dysfunction associated with or does it lead to negative affective states and vice versa?</p> <p>The current project aimed to improve the assessment of animal welfare by comparing the two common welfare assessment methods, using laying hens as the model species.</p>

Research	<p>In the first two experiments, Y maze tests were used to examine preferences for feed, social contact and a dustbathing substrate (sawdust). In the first experiment, the hens' choice for feed was higher than that expected if the choice was random for both those deprived of feed for 30 min or 4 h prior to testing. In contrast, the hens' choice for dustbathing substrate was not different from that expected if the choice was random for both groups of hens. In the second experiment, hens consistently chose feed over dust or social contact, irrespective of whether the birds were deprived of the resources before testing (3 h for feed and 12 h for dust or visual contact with birds).</p> <p>The third experiment in this project examined the importance of one aspect of the design of the preference test, quantity of the reward. The quantity of reward in a Y-maze preference test refers to the quantity of time the animal is allowed contact with its chosen resource. Overall, these results indicated that while there was no significant effect of the quantity of reward on choice behaviour, birds which received the intermediate reward quantity, when compared to the other treatments, showed evidence of reduced motivation to obtain dust as demonstrated by their tendency to choose social contact more often and their reduced speed of movement. The results of this preliminary study show that the quantity of reward has the potential to affect the choice behaviour of hens for peat moss and social contact and also that there are possible effects of preference for familiar birds on this choice behaviour.</p> <p>The fourth experiment examined the effect of a significant stressor, surgery and repeated blood sampling, on the biological functioning of laying hens. Biological functioning was measured using behaviour, productivity and plasma corticosterone variables. The behaviour and physiology data are presently being analysed and will be reported in June 2009 to the Poultry CRC as an addendum to this report.</p> <p>Experiment 5 in the project examined the effects of deprivation of a dust-bath or social contact of birds that either preferred for dust-bathing and social contact on biological functioning. The behaviour and physiology data are presently being analysed and will be reported in June 2009 to the Poultry CRC as an addendum to this report.</p>
Project Progress	Completed
Implications	<p>Scientists differ in their views on how animal welfare should be measured or judged, with three prominent concepts of animal welfare in the literature: the welfare of animals is judged on the basis of (1) how well the animal is performing from a biological functioning perspective; (2) affective states, such as suffering, pain and other feelings or emotions; and (3) the expression of normal or 'natural' behaviours. These different concepts or views on animal welfare lead to the different methodologies to assess an animal's welfare. This 'conceptual' uncertainty is one of the most obvious and challenging limitations for science in relation to its contribution to establishing and verifying animal welfare standards.</p> <p>The research conducted in this Poultry CRC project will contribute to a better understanding of the relationships between these concepts and methodologies. While the analyses in the final experiment in this project are presently incomplete, it is clear that further research is necessary to comprehensively test the underlying hypothesis in this research program. The outcome of this fundamental research endeavour is the integration of these welfare criteria and the development of a broader consensus on animal welfare methodologies to assist in reducing the interpretative differences in animal welfare science.</p>
Publications	Arnold, N.A., Ralph, C., Petherick, J.C., Hemsworth, P.H. (2007). The

	<p>choice behaviour of laying hens for feed and dustbathing. <i>Proceedings Australian Poultry Science Symposium</i>, 19, p.32.</p> <p>Arnold, N. A., Nash, J. A., Hemsworth, P. H. (2008). Preferences of laying hens for sawdust, feed and social contact. <i>Proceedings of the World's Poultry Congress</i>. Brisbane, Australia. 30th June – 4th July, 2008, p. 242.</p> <p>Barnett, J.L. Hemsworth, P. H, (2009). Welfare Monitoring Schemes: Using Research to Safeguard Welfare of Animals on the Farm. <i>Journal of Animal Welfare Science</i> (in press).</p> <p>Hemsworth, P. H., Widowski, T., Cronin, G. C., Barnett, J. L. (2008). Hen welfare – the issues and challenges. <i>Proceedings of the XXIII World's Poultry Congress</i>. 30th June – 4th July, 2009. Brisbane, Australia, p. 267.</p> <p>Laine, S. (2006). The effects of resource deprivation on the preferences and motivation of laying hens in a Y maze test. Honours thesis. University of Melbourne, Australia.</p> <p>Laine, S., Arnold, N.A., Hemsworth, P.H. (2007). Choice behaviour of laying hens: effects of deprivation of feed and dustbath substrate. <i>Proceedings Australian Poultry Science Symposium</i>, 19: 28-31.</p> <p>Laine, S.M., Cronin, G.M., Hemsworth, P.H., Petherick, J.C. (2008). The effects of quantity of reward on the choice behaviour of laying hens in a Y-maze preference test. <i>Proceedings of the 42nd Congress of the International Society for Applied Ethology</i>, Dublin, Ireland.</p> <p>Laine, S.M., Cronin, G.M., Petherick, J.C., Hemsworth, P.H. (2009). Does the quantity of reward in a Y-maze preference test affect hen choice and motivation? <i>Australian Poultry Science Symposium</i>, 20, 153-156.</p> <p>Widowski, T. M. and Hemsworth, P. H. (2008). Housing hens to suit their 'needs'. <i>Proceedings of the XXIII World's Poultry Congress</i>. 30th June – 4th July, 2009. Brisbane, Australia, p. 241.</p>
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