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Usefulness of Preference for Resources and Biological Functioning to Assess Animal Welfare

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2. Executive Summary

Science has a critical role in underpinning decisions on animal welfare standards. However, a current weakness in the scientific assessment of animal welfare and subsequently in establishing welfare standards for animals is that there are differing definitions of animal welfare.

Animal welfare is a state within an animal. It is not management procedures applied to the animal, nor features of the animal's environment, which may affect its welfare and most accept that animal welfare ultimately concerns animal feelings or emotions. However, there is uncertainty within science or at least the lack of a consensus position among scientists on the concept of animal welfare. Scientists utilize basically three concepts of animal welfare in judging the welfare of animals: how well the animal is performing from a biological functioning perspective; affective states, such as suffering, pain and other feelings or emotions; and the expression of normal or 'natural' behaviours.

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 concepts. While different concepts and consequently different methodologies may be used to assess animal welfare, 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 an intuitively aversive or rewarding condition reduces or improves animal welfare, respectively, on the basis of the measures of different concepts of animal welfare. The objective of the research conducted in this project was to examine the validity of the welfare criteria through the second approach: that is, test the hypothesis that restriction of a resource or behaviour that animals are highly motivated to access or perform, respectively, leads to disruption to biological function, such as occurrence of abnormal behaviour, increased stress and poor growth and health.

This experiment examined the effects of social restriction of pigs that were classified as strongly socially preferred or moderately socially preferred on disruption in biological function. It was found that when pigs were socially restricted by limiting their tactile contact or removing their visual and tactile contact with neighbouring pigs, the preference classification of the pig affected saliva cortisol concentrations: strongly socially preferred pigs had higher saliva cortisol concentrations.

This finding suggests that strongly socially preferred pigs are so highly motivated to have full tactile contact with pigs that they experience stress when restricted of tactile contact with pigs. An alternative but less likely explanation is that housing these pigs in individual pens either with limited tactile contact or no visual and tactile contact with neighbouring pigs leads to unresolved aggression in unsuccessfully attempting to establish a dominance relationship.

Surprisingly, while there are numerous examples in the literature in both pigs and other species where housing, handling or husbandry effects that have affected cortisol concentrations have also affected behaviour, growth or reproduction, there was no evidence in the present experiment that preference classification affected behaviour or live weight gain.

Nevertheless, these results together with limited evidence from several other studies indicate that restriction of a resource that animals are highly motivated to access leads to disruption to biological function, such as occurrence of abnormal behaviour, increased stress and poor growth and health. These results should provide confidence to both scientists studying impacts on animal welfare and policy makers utilizing science to inform animal welfare standards and recommendations that approaches examining biological function and animal preferences, providing the methodology is sound, provide the best current assessment of animal welfare. However, where the opportunity exists, any argument for impaired welfare due to restriction of a resource or behaviour would be strengthened by both evidence that animals are highly motivated to access the resource or perform the behaviour, respectively, and evidence of disruption to biological function.

3. Background to Research

Animal welfare is an increasingly contributing perspective in society, strongly influencing the acceptability of various farm animal management options. There has been and still remains a clear priority to avoid animal suffering. Suffering is a term in common use to denote strongly and intense negative or noxious subjective or emotional mental experiences such as anxiety, fear and pain (Mellor *et al*., 2009). Furthermore, 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). Indeed some have suggested that it is widely accepted that "good welfare is not simply the absence of negative experiences, but rather is primarily the presence of positive experiences such as pleasure" (Boissy et al., 2007).

Science has a critical role in underpinning decisions on animal welfare standards. Failure to assure key stakeholders, particularly the consumer and the general public, that the welfare standards for farm animals are underpinned by sound science has the potential to adversely influence the profitability and viability of animal industries such as the poultry industries by affecting specific practices such as current as well as new housing and husbandry.

However, a current weakness in the scientific assessment of animal welfare and subsequently in establishing welfare standards for animals is that there are differing definitions of animal welfare. There is uncertainty within science (Sandoe *et al*., 2004; Barnett and Hemsworth, 2009) or at least the lack of a consensus position among scientists (Fraser, 2003; 2008) 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.

The first concept, which is often called the biological functioning concept, is underpinned by 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, 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. Thus assessing biological functioning involves a broad examination of the behavioural, physiological, health and fitness responses of animals in reaction to condition under study on the basis that difficult or inadequate adaptation will generate welfare problems for animals.

The second concept, sometimes called the affective states or mental functioning concept, defines animal welfare in terms of emotions and thus it 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 described the argument 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 emotions to motivate behaviour to meet these needs. Emotions are classically described through a behavioural component (a posture or an activity), an autonomic component (visceral and endocrine responses) and a subjective component (emotional experience or feeling) (Dantzer, 1988). Thus measuring preferences of animals, using preference tests, aversion learning and behavioural demand tests (Dawkins, 1980; Matthews and Ladewig, 1994), has been used by scientists to assess animal welfare 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.

While not well enunciated, the third concept promotes the principle that animals should be allowed to express their normal behaviour, which for some means that animals should be raised in 'natural' environments and allowed to behave in 'natural' ways. A difficulty with this concept is attributing actual suffering when the expressions of certain behaviours is prevented or absent. There is also some emphasis on behavioural indicators of poor coping such as fearfulness, aggression and stereotypies (EFSA, 2005), but these are generally indicators used in the other two main approaches.

This 'conceptual' uncertainty is an obvious limitation for science in relation to its contribution to establishing and verifying animal welfare measures and standards. Notwithstanding the uncertainties surrounding the definition and assessment of animal welfare, current related issues include the need for both research and field measures of animal welfare and the ability to benchmark animal welfare outcomes.

These different concepts or views on animal welfare can lead scientists to use different criteria or methodology in assessing an animal's welfare. For some animal welfare issues, such as floor space effects, there is at least some degree of agreement arising when utilising different criteria or methodology. However, disagreement over these welfare concepts or criteria, especially when criteria or interpretations conflict, lead to debates concerning animal welfare and the varying interpretations of the effects of confinement on laying hens and gestating sows are obvious examples of the consequences of disagreement on the concept of animal welfare.

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 concepts (Barnett and Hemsworth, 2009). While different concepts and consequently different methodologies may be used to assess animal welfare, the validity of the welfare criteria can be tested in several ways (Barnett and Hemsworth, 2009): first, with the finding that there are correlations between independent measures of different concepts of animal welfare; and second, with the finding that an intuitively aversive or rewarding condition reduces or improves animal welfare, respectively, on the basis of the measures of different concepts of animal welfare. The objective of the research conducted in this project was to examine the validity of the welfare criteria through the second approach: that is, test the hypothesis that restriction of a resource or behaviour that animals are highly motivated to access or perform, respectively, leads to disruption to biological function, such as occurrence of abnormal behaviour, increased stress and poor growth and health.

Previous research has shown that pigs vary in their motivation for social contact relative to feed in Y-maze preference tests (Hemsworth et al., 2011). Therefore, using two highly preferred resources and resources for which individual pigs may differ in their preference, provides an excellent model to study the effects of restriction of preferred resources on biological function. Consequently, the approach was taken in this project to test the hypothesis that deprivation of social contact in pigs that highly prefer social contact results in disruption to biological function.

4. Objectives of the Research Project

The objective of this project was to determine the validity of the criteria arising from the two main welfare concepts, biological functioning and affective states, by examining the hypothesis that restriction of a resource or behaviour that animals are highly motivated to access or perform, respectively, leads to disruption to biological function, such as occurrence of abnormal behaviour, increased stress and poor growth and health. This finding would assist in reducing the interpretative differences in animal welfare science, a development that is critical to the welfare debate and informing policy decisions that are acceptable to the community.

Aims

This project examined the effects of deprivation of social contact in pigs that highly prefer social contact on their biological function.

5. Introductory Technical Information

The review by Hemsworth (2013) is utilized here to provide the rationale for these two concepts of animal welfare, biological functioning and affective states, and their resultant methodologies is as follows.

Biological Functioning

The biological functioning concept has as its underpinning rationale that difficult or inadequate adaptation will generate welfare problems for animals. Broom (1986, 1996) defines the welfare of an animal as "its state as regards its attempts to cope with its environment". The "state as regards attempts to cope" refers first, to how much has to be done in order to cope with the environment and includes biological responses such as the functioning of body repair systems, immunological defences, physiological stress responses and a variety of behavioural responses, and second, to the extent to which these coping attempts are succeeding. These behavioural and physiological responses include abnormal behaviours, such as stereotypies and redirected behaviours, and the stress response, respectively, while the success of the coping attempts are measured in terms of lack of biological costs, such as adverse effects on the animal's ability to grow, reproduce and remain healthy and injury-free (i.e., fitness effects).

The responses to stress are integral to the ability of an animal to cope and, in turn, to the welfare of the animal. The behavioural and physiological adaptive responses are utilized by individuals to cope with challenges (Broom, 1986, 1996; Broom and Johnson, 1993; Moberg, 2000; Barnett, 2003). Marked challenges may overwhelm an individual's capacity to adapt and lead to its death. However, although less severe challenges would not be fatal, they can still have significant biological costs, leading for example to impaired growth, reproduction and health, which in turn may result in welfare problems for the animal. In other words, it is the biological cost of stress that is the key to understanding the associated welfare implications (Moberg, 2000; Barnett, 2003). How well an animal is coping with the challenges it faces will be reflected in the normality of its biological functioning, and severe risks to welfare will be associated with the most extreme coping attempts.

Some have criticised this concept of animal welfare on the basis that it does not adequately include emotions. However, this would only be valid if emotions are independent of other biological processes, but this is unlikely since the mental state of an animal is an integral component of its biological state (Dantzer and Mormede, 1983). Emotional responses are produced in the limbic system, which projects to several parts of the brain, including those involved in the initiation and maintenance of the stress response, thus explaining why emotional insults activate a stress response (Kaltas and Chrousos, 2007).

In conclusion, how well an animal is coping with the challenges it faces will be reflected in the normality of its biological functioning, and difficult or inadequate adaptation will affect the fitness of the animal through a range of long-lasting behavioural and neuroendocrine responses. These behavioural and physiological responses include abnormal behaviours, such as stereotypies and redirected and displacement behaviours, and the stress responses including those involving both the sympathetic-adrenal-medullary and the hypothalamic-pituitary-adrenal axes, respectively, while the biological cost includes adverse effects on the animal's ability to grow, reproduce and remain healthy and injury-free.

Affective States

The second concept, often called the affective state or 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 that provide more flexible means for motivating behaviour to meet these needs. Thus the central argument is that although natural selection has shaped animals to maximize their reproductive success, this is achieved by proximate mechanisms involving affective states (pain, fear, separation distress, etc.) which motivate behaviour (Fraser, 2003).

While emotions are poorly defined, impossible to measure directly, and difficult to measure indirectly (Duncan, 2005), there has been a substantial growth over the last two decades in the literature on this topic of emotions (Panksepp, 1998, 2005; Denton *et al*., 2009). There are numerous definitions of emotions in the literature but an emotion can be defined as an intense but short-lived affective response to an event, which is associated with specific body changes and thus is classically described through a behavioural component (a posture or an activity), an autonomic component (visceral and endocrine responses) and a subjective component (emotional experience or feeling) (Dantzer, 1988). Denton *et al*. (2009) consider two classes of emotions. Primordial emotions are viewed as the subjective element of the instincts which are the genetically programmed behaviour patterns which participate in maintaining homeostasis. They include thirst, hunger for air, hunger for food, pain and hunger for specific minerals etc. There are two constituents of a primordial emotion, the specific sensation which when severe may be dominant, and the compelling intention for gratification by a consummatory act. They may dominate the stream of consciousness, and can have plenipotentiary power over behaviour. These primordial emotions are predominantly driven by sensors detecting deviation from normal within the body (interoceptors), and this class of emotions contrasts with another class of emotions which are most often fired by the distance receptors (exteroceptors) within the eyes, ears and the nose. These distance receptor evoke emotions, like rage, fear, hate, envy, happiness, playfulness, affection, anxiety, depression and disgust, are those to which the term emotion is most commonly applied and they are most often determined by situational perception. When emotions reach both a commanding specific sensation and a compelling specific intention, they motivate animals to engage in behaviours for which there is some strong evolutionary benefit (Denton *et al*., 2009).

Animal emotions have in the past been considered inaccessible to scientific investigation because they have been described as human subjective experiences or even as illusory concepts outside the realm of scientific inquiry (Panksepp, 1998). Given the very nature of emotional self-experience, there is no way to know if animals experience emotions similar to humans (Boissy *et al.* (2007). However, behaviour, structure, and brain chemistry are similar in humans and in a large number of animal species: other mammals are attracted to the same environmental rewards and drugs of abuse as humans; human emotions appear to be dependent on very similar sub-cortical brain systems situated in deep brain regions where evolutionarily homologous "instinctual" neural systems exist; and artificial activations of the deep brain systems that promote emotional actions are liked and disliked by animals, as measured by a host of approach and avoidance responses (see review by Panksepp, 2005).

Preferences can be measured as a means to determine what resources are important to an animal. Initial use of preference methodologies appeared in the literature in the 1970s (e.g., Hughes and Black, 1973; Dawkins, 1976). 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 animals. Essentially, these tests are designed to answer the question *"what is the relative importance of this feature for this animal?".*

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-immobilization, a procedure in which a pulsed, low-voltage current can be used to immobilize 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. It was found over repeated trials that sheep showed increasing avoidance of a location in which they were restrained with electro-immobilization 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 not absolute, some have argued that in addition to establishing what an animal prefers, it is important to understand the strength of the preference (Dawkins, 1983; Matthews and Ladewig, 1994). To address the question of the strength of an animal's preference, experiments have incorporated varying levels of cost (e.g., work effort, time and relinquishing a desirable resource) associated with gaining access to a resource or avoiding aversive stimulation. For example, 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. Food can be considered as the "gold standard" in preference testing (Matthews and Ladewig, 1994), and therefore is generally expected to produce a maximal response or preference.

Furthermore, Dawkins (1983) suggested that quantitative measures of the importance of resources for animals can be derived from measures of demand elasticity. Consequently, 'behavioural demand' studies, using operant conditioning techniques in which the animal must learn to perform a response, such as pecking at a key or pushing through a weighted door, to gain access to a resource, have been used to study the animal's level of motivation to access or avoid the situation being tested For example, Matthews and Ladewig (1994) studied the behavioural demand functions of pigs for the resources 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. It was found that while the demand for opening the pen door was highly elastic (i.e. the willingness of the pigs to access the resource declined as the effort increased), the demand for food was inelastic and the demand for social contact was intermediate.

While it seems likely that animals will avoid aversive stimulation and choose positive stimulation, preference and motivation testing have generated considerable debate relating to conceptual and methodological difficulties (see Nicol et al., 2009; Fraser and Nicol, 2011).

For example, familiarity with a resource may affect choice, a choice at a point in time may not reflect interactions of different motivational states over time, a positive resource may remind the animal of a resource that it may not otherwise miss, the choices may not be within the animal's cognitive capacity and vigilance behaviour may be misinterpreted as a choice.

As with biological functioning, clarifying the conceptual link between animal preferences and animal welfare is difficult. Nevertheless, as argued by a number of authors (e.g. Fraser and Matthews, 1997; Widowski and Hemsworth, 2008), while studies of motivation can provide evidence that the performance of some behaviours (or preferences) may be important to the animal, additional evidence, particularly on the occurrence of abnormal behaviour, stress physiology and health, are necessary to provide a more comprehensive assessment of the impact of restriction on animal welfare.

Assessing Animal Welfare

Basically scientists have used two main concepts in studying animal welfare, biological functioning and affective states, and these studies require the disciplines of animal behaviour, immunology, neurophysiology, psychology, stress physiology, and veterinary science.

Assessment of animal welfare on the basis of biological functioning involves examining how well the animal is performing from a biological functioning perspective, that is, how well the animal has adapted to its environment. Assessment on the basis of affective states (emotions) involves examining both negative states, such as fear, pain, hunger and distress, and positive ones, such as satiety, contentment and playfulness (Mellor *et al.* 2009). This scientific uncertainty in relation to animal welfare concepts does not necessarily diminish the robustness of the research utilising criteria or methodologies promulgated by these different concepts (Barnett and Hemsworth, 2009).

While different concepts and consequently different methodologies may be used to assess animal welfare, the validity of the welfare criteria can be tested in several ways (Barnett and Hemsworth, 2009): first, with the finding that there are correlations between independent measures of different concepts of animal welfare; and second, with the finding that an intuitively aversive or rewarding condition reduces or improves animal welfare, respectively, on the basis of the measures of different concepts of animal welfare. Indeed, there is limited evidence of the relatedness of these concepts (Nicol *et al*., 2009, 2011; Stevens *et al*., 2009): that is, animals are motivated to choose those resources or behaviours that maintain normal biological functioning in terms of behaviour, physiology and health. Further research on the relatedness of these three concepts is required.

Research Methodology

Facilities

This study was conducted at the Department of Primary Industries research facility at Werribee, Victoria and commenced in March 2012 and concluded in July 2012. The pigs were housed and tested in a steel-sided, mechanically ventilated building providing natural and artificial lighting, for approximately 9 hours per day, lighting the housing and testing areas. A purpose built Y-maze was used to study the choice behaviour of the pigs for the two resources. The maze was located adjacent to the pig housing rooms in the aisle way. Pigs individually entered the start-box (2.0 x 1.5 m, length x width), where the test pigs had visual contact with the two short arms of the Y maze (each 2.0 × 1.5 m) through a mesh gate (start gate) that opened into the long arm of the maze (3.0 ×1.5 m) (see Figure 1).

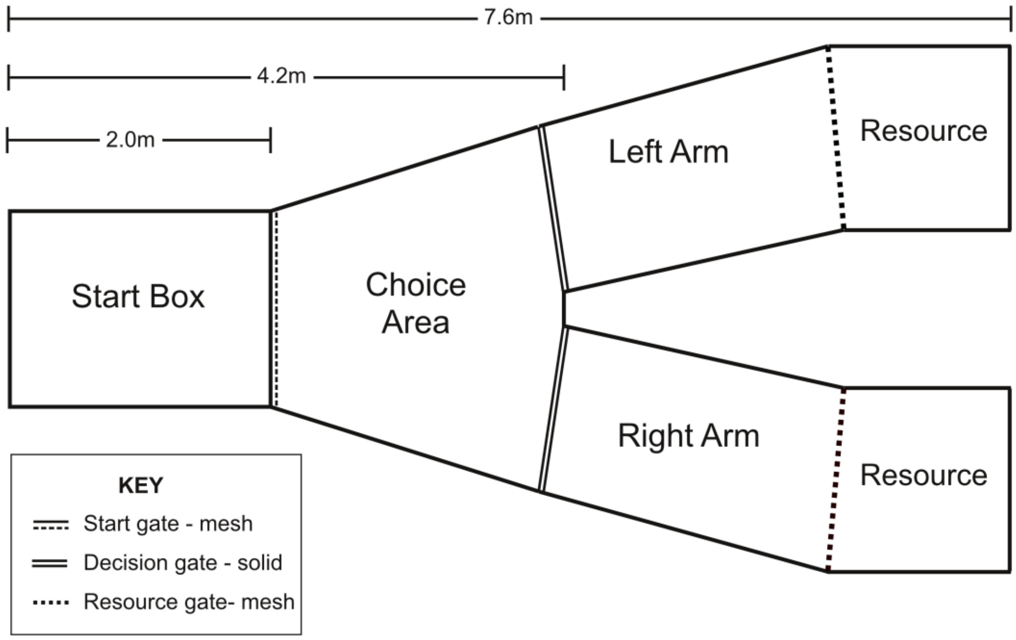

Figure 1: Diagram of Y-maze testing apparatus

Animals and Treatments

A total of 44 pre-pubertal gilts, in two replicates (22 per replicate) were used in the study. The gilts were crossbred (Large White x Landrace) and were 12 weeks old on arrival. They were sourced from a commercial piggery in Kialla, Victoria. On arrival the pigs were weighed and ear tagged for individual identification, then group housed on rice hulls in their established group with overhead heat lamps. They had access to *ad libitum* water and access to *ad libitum* feed (calculated at approx 1.7 kg per pig of a commercial grower pelleted diet. They were given one week to acclimatize to their new surroundings and during this time they received human contact only during routine feeding and maintenance.

This experiment consists of two parts. The first part examined the pigs preferences for human contact or social contact and then the second part examined normal biological functioning of individual pigs when deprived of their more preferred resource, social contact or human contact. By examining the effects of deprivation of the more preferred resource on biological functioning, this experiment studied the relationship, if any, between these two methodologies of animal welfare assessment.

Part 1 - Preferences

Training Phase

The choice behaviour of 18 (36 in total over two replicates) grower pigs for human or social contact was studied in a series of Y maze trials over 12 days while the pigs were restricted of both human and social contact.

After a week of acclimatization 18 animals were randomly selected and moved into individual pens (six pens/room in 3 rooms, pen size approx 0.75 x 2m) with human and social contact restrictions. Pigs were provided with a bedding of rice hulls and had access to water *ad libitum*. Pigs were fed daily a commercial grower pelleted diet (Barastoc Ultrawean 250, 14.5MJ/kg, 20% Crude protein) at 0830h. Feed was weighed out daily and feed refusals were collected prior to feeding allowing for daily intake of food for each pig to be calculated. The pigs were weighed on arrival, at the end of Y maze testing and at the completion of the experiment. The remaining 4 pigs were used as the stimulus pigs for the social contact resource in the Y-maze. These spare pigs were group housed on rice hulls with *ad libitum* access to water and feed.

During the first week of training, which was conducted in the mornings at least 30 minutes after feeding, the pigs were daily introduced individually to the empty Y maze for 2 min in 2 sessions over two days to familiarize them with the apparatus. During the next three days of the training week, the pigs were trained to use the Y maze, with the two resources in the arms, a human sitting on stool in one arm vs. 2 stimulus pigs in the other arm. The pairing of arm to resource remained consistent for each pig in all training and testing trials over the entire study. Over the next 3 days the pigs were run through a series of training tests whereby only one arm of the Y maze containing a resource was open at a time, they were given 2 min within that arm then returned to the start box and then ran back through the test again with the opposite arm and resource open and given access for 2 min within that arm. This training testing was done over 3 days, twice a day morning and afternoon. The order of testing pigs was randomly allocated as was which arm would be opened first or second. This way the pigs learnt that the two stimuli would always be in the same arms. The resource position was randomly assigned to each pig.

Treatment and Testing Phase

In a 2 week testing phase, choice behaviour was studied in daily tests, in which animals could access either resource via one arm. Each test involved introducing pigs individually in a starting box at the start of the long arm of the Y maze. Following 5 sec in the starting box, the pig was released into the Y maze at the junction of the two arms, with the resources in the two arms (Social contact vs. human contact) and once the pig selects and enters one of the arms, a gate on the other arm was closed and the pig then remained within the arm with the chosen resource for 2 minutes.

Each pig was tested daily over 12 consecutive days during which deprivation of social and human contact was imposed.

The time taken to exit the start box, the resource chosen and the time taken to choose the resource were recorded.
After the Y maze testing phase was completed each animal was put through a stationary human test. An arena was set up in a vacant pen (9.6m2). Pigs were removed individually from their home pens and introduced into the arena. After a 2 min-familiarization period alone in the arena, the unfamiliar human stimulus entered and positioned themselves at the mid-point of the wall adjacent the entrance where they remained stationary, the test starts once the human has reached the test position. Lines were marked on the floor 0.5m around the human stimulus prior to the test. During the next 3 min, the following observations were made on each animal, the time taken to approach within 0.5 m of the human, the total time spent within 0.5 m of the human, the time to the first physical interaction with the human and the number of physical interactions with the human such as biting and licking the human's apparel (a bout criterion interval of 5s was used to separate one bout from another).

The human stimulus verbally relayed the pig's responses during the test and an overhead camera recorded the tests for future reference.

Part 2 – Biological Functioning
From the 18 (36 in total over two replicates) pigs preference tested, 8 (16 over two replicates) were selected on the basis of their preferred choice for Part 2 of the experiment. 4 (8) highly socially preferred pigs and 4 (8) least socially preferred pigs. The 8 (16) pigs selected for Part 2 were randomly allocated to new individual pens, 4 pigs per room, over 2 rooms.

The 8 selected pigs, 4 identified as highly socially preferred and 4 least socially preferred were then studied when deprived of social contact (tactile and visual restriction with pigs).

Week 1 was the pre-treatment period in which the pigs were individually housed in pens in which tactile and visual contact with a neighbouring pig was available through a wire-mesh partition.

Weeks 2, 3, and 4 were the treatment period in which the pigs were individually housed in pens with no tactile or visual contact with a neighbouring pig was available.

Stress Physiology
Saliva Cortisol
A total of four saliva samples were collected from each pig in each of weeks 1, 2 and 4 in Part 2. A single saliva sample was collected from each pig on each of 4 days (Mondays to Thursdays) in each of these 3 weeks commencing at 13:00 h and was collected within 2 min of the technician approaching to animal to collect saliva in order to minimize effects of handling on saliva cortisol concentrations (Broom and Johnson, 1993). The saliva samples were collected by placing a cotton bud (Salivettes, Sarstedt Australia, South Australia, Australia) attached to a cable tie in the pigs mouth allowing the pig to chew on it thus sufficiently moistening the cotton bud with saliva. Samples were centrifuged and the saliva collected and frozen at -18C until subsequently analysed.

Plasma Cortisol

A 10ml blood sample was collected from each pig via jugular venipuncture (10-mL lithium-heparinised tubes (BD Vacutainer® BD, Belliver Industrial estate, Plymouth, UK)) when pigs were restrained with a snout snare in weeks 1, 2, and 4. A single blood sample was collected from each pig on one day (Friday) in each of these 3 weeks commencing at 13:00 h and was collected within 2 min of the technician snaring the animal in order to minimize effects of handling on saliva cortisol concentrations (Broom and Johnson, 1993). The sample was collected into a heparin coated tube within a 3min window thus providing a sample at rest. The blood samples were centrifuged and the plasma collected and frozen at -18C until assayed for total and free cortisol.

Cortisol Assays

Total plasma and saliva concentrations of cortisol were quantified using commercial radioimmunoassay kits (Diasorin Australia Ltd. NSW). The sensitivity of the assay was 3.5nmol/L. Mean intra assay variation for medium (32.75nmol/L) and high (71.2nmol/L) plasma samples were 0.9% and 3%, respectively.

Haematology

In week 4 a second 10ml blood sample was collected from each pig via jugular venipuncture (10-mL lithium- heparinised tubes (BD Vacutainer® BD, Belliver Industrial estate, Plymouth, UK)) when pigs were restrained with a snout snare as pigs were also sampled for plasma cortisol. This whole blood sample was kept cool on ice, transported to an Australian commercial laboratory and the absolute numbers of neutrophil and lymphocyte cells were measured on individual pig samples in a CellDyn 3700 autoanalyzer (Abbott Diagnostic Division, Abbott Park, IL) and the neutrophil-to-lymphocyte ratio was calculated for each pig.

Live Weight Gain

Daily feed intake was recorded throughout the experiment and the pigs were weighed on arrival, at the completion of PART 1 and at the completion of PART 2. Pigs were fed a commercial grower pelleted diet (Barastoc MP Pig 1300, 14.0MJ/kg, 18.1% Crude protein) once daily at 0830 h. Feed residues were collected daily.

Behaviour

Cameras were mounted over the pens to record the behaviour of the pigs one day a week over 4 weeks from 07:00-15:00 h. From the video records, instantaneous sampling at 15 min intervals was used to record pig postures, lying, standing, sitting and kneeling.

Also pig behaviours were recorded, nosing the floor, nosing pen fittings, nosing neighbouring pig, feeding, drinking, walking or being idle.

Statistical Analyses

Repeated Measures General Linear Model (SPSS 19.0, SPSS Inc., Chicago, Illinois, USA) was used to examine treatments effects on saliva and plasma cortisol concentrations over the 4 week study period in Part 2. The mean of the concentrations of four saliva samples collected from each pig in each of the 3 weeks was used in this statistical analysis. Univariate General Linear Model (SPSS 19.0, SPSS Inc., Chicago, Illinois, USA) was used to examine treatment effects on behavioural data, body weights and white blood cell counts. Where required, data were log transformed to meet the assumption of normality.

6. Discussion of Results

Part 1- Preference Testing

Choice Behaviour

The choice behaviour indicates that while pigs were overall more motivated to access social contact with other pigs than human contact (average proportion of trials in which social contact was chosen was 0.818), there was considerable individual variation between pigs (Figure 2, (standard deviation of 0.221)). This supports previous findings of substantial variation in the choice behaviour of individual pigs for social contact and feed (Hemsworth, 2009).

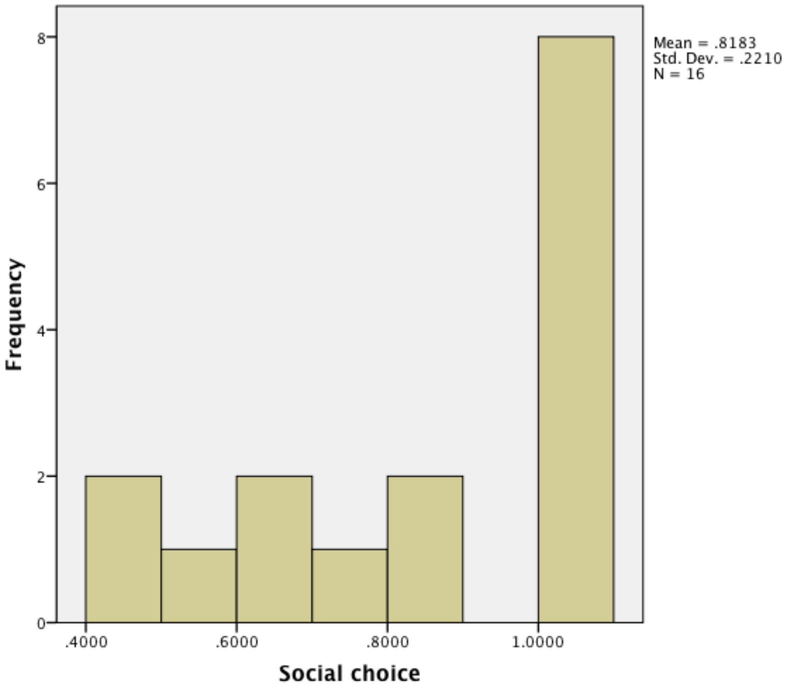

Figure 2: Histogram showing the proportion of choice trials in which individual pigs chose social contact.

Based on the overall choice behaviour of the 36 pigs, 8 were selected as strongly socially preferred on the basis of choosing social contact in 100% of the trials and 8 pigs were selected as moderately socially preferred on the basis of choosing social contact in an average of 63% of trials.

Part 2 – Biological functioning

Physiology

As shown in Table 1, strongly socially preferred pigs had higher saliva cortisol concentrations, but not plasma cortisol concentrations, than moderately socially preferred pigs when housed in individual pens in which tactile and visual contact with a neighbouring pig was available through a wire-mesh partition (Week 1 in Part 2) and individual pens in which no with no tactile or visual contact with a neighbouring pig was available (Weeks 2, 3, and 4 in Part 2).

Table 1: The effects of preference classification on saliva and plasma cortisol concentrations in Part 2. Estimated marginal means (and standard errors) presented.

Variable	Preference level for social contact		P values
	Strong	Moderate	
Cortisol concentration (nmol/L)			
Saliva	19.6 (2.25)	8.8 (2.10)	**0.004**
Plasma	69.0 (12.72)	58.9 (11.90)	0.571

There was no (P>0.05) effects of preference classification on absolute numbers of neutrophil and lymphocyte cells or neutrophil-to-lymphocyte ratio (Table 2).

Table 2: The effects of preference classification on haematology in Part 2. Estimated marginal means (and standard errors) presented.

Variable	Preference level for social contact		P values
	Strong	Moderate	
Haematology			
Neutrophils (absolute numbers)	4.43 (0.827)	4.19 (0.555)	0.539
Lymphocytes (absolute numbers)	12.97 (2.827)	14.32 (1.720)	0.303
N/L ratio	0.31 (0.065)	0.33 (0.066)	0.596

Live Weight Gain

There was no (P>0.05) effect of preference classification on live weight gain (Table 2).

Table 3: The effects of preference classification on live weight gain in Part 2. Estimated marginal means (and standard errors) presented.

Variable	Preference level for social contact		P values
	Strong	Moderate	
LW gain (kg)	35.1 (3.06)	34.7 (2.87)	0.926

Behaviour

There were no significant (P>0.05) differences in home pen behaviour between the two preference groups. All pigs spent the largest proportion of their time in the home pen lying down (overall average 22.7% of all observations) and idle or not performing any specific behaviours (overall average 22.4% of observations).

7. Implications & Recommendations

This experiment examined the effects of social restriction of pigs that were classified as strongly socially preferred or moderately socially preferred on disruption in biological function. In addition to behaviour, another biological response that is a focus in studying biological function is the physiological stress response, since the responses to stress are integral to the ability of an animal to cope and, in turn, to the welfare of the animal (Broom, 1986, 1996; Broom and Johnson, 1993; Moberg, 2000; Barnett, 2003). In Part 2 when all pigs were socially restricted, the preference classification of the pig affected saliva cortisol concentrations over the 4 weeks in Part 2: strongly socially preferred pigs had higher saliva cortisol concentrations over the 4 weeks. However, a surprising aspect of this finding is that while saliva cortisol concentrations were higher in the strongly socially preferred pigs in Weeks 2 and 4 of Part 2 when housed in individual pens in which no tactile or visual contact with a neighbouring pig was available, saliva cortisol concentrations were also higher in Week 1 in these pigs when housed in individual pens in which tactile and visual contact with a neighbouring pig was available through a wire-mesh partition. If social contact is an important resource for these strongly preferred pigs to the extent that biological function would is disrupted when they are socially deprived, one would expect effects on stress in Weeks 2 and 4 when no tactile or visual contact was possible with a neighbouring pig, and less so in Week 1 when tactile or visual contact was possible with a neighbouring pig. It is possible therefore, that strongly socially preferred pigs are so highly motivated to have full tactile contact with pigs that even restriction of tactile contact with pigs is stressful. An alternative but less likely explanation is that housing these pigs in individual pens either with limited tactile contact or no visual and tactile contact with neighbouring pigs leads to unresolved aggression in unsuccessfully attempting to establish a dominance relationship. Barnett and colleagues in a series of experiments (see review by Barnett et al., 1991) found that the design of the stall division for pregnant gilts in stalls or tether stalls affected both aggression and cortisol concentrations. For example, tether stall divisions in which vertical bars separated neighbouring pigs resulted in a chronic stress response, whereas, if the bars were covered in steel mesh, the free cortisol concentrations were similar to those in group-housed pigs. Barnett et al. (1991) concluded that the design of stall division may lead to unresolved aggression between neighbouring pigs and consequently stress.

In contrast to effects on saliva cortisol, there were no effects of preference classification on plasma cortisol concentrations. While saliva cortisol is considered a reliable measure of free plasma cortisol (Levine et al., 2007), saliva cortisol sampling was more comprehensive in the present experiment than that of plasma cortisol concentrations (four times per week in Weeks 1, 2, and 4 vs. weekly in Weeks 1, 2 and 4) and consequently may have provided a more accurate sample of the activation on the hypothalamic-pituitary-adrenal axis.

While there were preference classification effects of saliva cortisol concentrations, there were no effects on behaviour and live weight gain. There are numerous examples in the literature in both pigs and other species where housing, handling or husbandry effects that have affected cortisol concentrations have also affected behaviour, growth or reproduction (handling of pigs, poultry and dairy cows (see review by Hemsworth and Coleman, 2011), housing of pigs and poultry (see reviews by Barnett et al., 2001; Barnett and Hemsworth, 2003), mulesing of lambs (see review by Edwards, 2012). However, there are also examples where prolonged elevations in glucocorticoid concentrations have not been accompanied by adverse effects on fitness variables. In 2 experiments, Barnett et al. (1989) found that pregnant gilts housed in stalls, with either vertical bars or wire mesh on the front section of stall divisions, showed a moderate but significant increase in basal free cortisol concentrations compared with pigs housed in groups, but this increase was markedly less than that of pigs housed in tethers. Furthermore, whereas glucose concentrations were elevated, indicative of a metabolic cost, in gilts housed in tethers, no increase was evident in gilts in the two stall treatments in either experiment. Indeed, Barnett and Hemsworth (1990) argued that the results of handling and housing experiments conducted by the group show that detrimental consequences on growth rate, sexual behaviour or pregnancy rate generally occur when there are increases in free corticosteroids of greater than 40%. However, the average change in saliva cortisol concentrations of the pigs in strongly socially preferred classification in the present experiment well exceeded 40% of those in the moderately socially preferred classification.

An obvious difficulty in this experiment and in previous work (Hemsworth, 2009) is that the use the Y-maze procedure, a competitive choice test, provides a relative assessment of preference for the two resources under study (in this experiment, social contact compared to human contact) rather than an absolute preference. In the design of the study used here in Part 2 to examine deprivation of social contact, differences between the two preference classifications may not have differed as expected in absolute terms since the pigs were selected on relative preference, that is choice of social contact relative to human contact.

Nevertheless, these results together with limited evidence from several other studies (Stevens et al., 2009; Nicol et al., 2009, 2011) indicate that restriction of a resource that animals are highly motivated to access leads to disruption to biological function, such as occurrence of abnormal behaviour, increased stress and poor growth and health. Stevens et al. (2009) in a factorial design examined the effects of deprivation of feed (70% of estimated voluntary feed intake) or social contact (deprived of visual and tactile social contact) on pigs classified as either feed preferred or social preferred. There was a significant interaction between the main effects of deprivation and preference on live weight: when deprived of feed, pigs in the feed preferred classification weighed less but when deprived of social contact, pigs in the social preferred classification weighed less. There was also a tendency for an interaction between main effects on free cortisol concentrations: when deprived of feed, the pigs in the feed preferred classification had higher cortisol concentrations while the social preferred classification had higher cortisol concentrations when deprived of social contact. Nicol et al. (2009) examined the relationship between choice behaviour of laying hens for several differing environments (wire-mesh floor; wood shavings floor; and peat floor with perch and nest box) and the behavioural and physiological responses of these hens when housed in the longer term in these environments. Biological responses associated with choice included lower body temperature, blood glucose, heterophil:lymphocyte ratio and behavioural response to novelty, and greater feed digestibility and self-grooming.

These present findings together with those of several recent experiments indicate that restricting animals of a resource that they are highly motivated to access disrupts biological function on the basis of the display of abnormal behaviour, increased stress and poor growth and health. These results should provide confidence to both scientists studying impacts on animal welfare and policy makers utilize science to inform animal welfare standards and recommendations that approaches examining biological function and animal preferences, providing the methodology is sound, provides the best current assessment of animal welfare. However, where the opportunity exists, any argument for impaired welfare due to restriction of a resource or behaviour would be strengthened by both evidence that animals are highly motivated to access the resource or perform the behaviour, respectively, and evidence of disruption to biological function.

8. Intellectual Property

Information generated at this stage of the RD&E process, while creating intellectual property value and will be distributed via publications, does not lead to patentable outcomes.

9. Technical Summary - Summary of Information Developed as a Part of the Research, e.g. Discoveries in Methodology, Equipment Design, etc.

There has been and still remains a clear priority to avoid animal suffering. Suffering is a term in common use to denote strongly and intense negative or noxious subjective or emotional mental experiences such as anxiety, fear and pain.

A current weakness in the scientific assessment of animal welfare and subsequently in establishing welfare standards for animals is that there are differing definitions of animal welfare. There is uncertainty within science or at least the lack of a consensus position among scientists 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.

The first concept, which is often called the biological functioning concept, is underpinned by the animal welfare definition that "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, 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. Thus assessing biological functioning involves a broad examination of the behavioural, physiological, health and fitness responses of animals in reaction to condition under study on the basis that difficult or inadequate adaptation will generate welfare problems for animals.

The second concept, sometimes called the affective states or mental functioning concept, defines animal welfare in terms of emotions and thus it emphasizes reductions in negative emotions, such as pain and fear, and increases in positive emotions such as comfort and pleasure. Emotions are classically described through a behavioural component (a posture or an activity), an autonomic component (visceral and endocrine responses) and a subjective component (emotional experience or feeling). Thus measuring preferences of animals, using preference tests, aversion learning and behavioural demand tests, has been used by scientists to assess animal welfare 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.

While not well enunciated, the third concept promotes the principle that animals should be allowed to express their normal behaviour, which for some means that animals should be raised in 'natural' environments and allowed to behave in 'natural' ways. A difficulty with this concept is attributing actual suffering when the expressions of certain behaviours is prevented or absent. There is also some emphasis on behavioural indicators of poor coping such as fearfulness, aggression and stereotypies, but these are generally indicators used in the other two main approaches.

This 'conceptual' uncertainty is an obvious limitation for science in relation to its contribution to establishing and verifying animal welfare measures and standards. Notwithstanding the uncertainties surrounding the definition and assessment of animal welfare, current related issues include the need for both research and field measures of animal welfare and the ability to benchmark animal welfare outcomes.

These different concepts or views on animal welfare can lead scientists to use different criteria or methodology in assessing an animal's welfare. For some animal welfare issues, such as floor space effects, there is at least some degree of agreement arising when utilising different criteria or methodology. However, disagreement over these welfare concepts or criteria, especially when criteria or interpretations conflict, lead to debates concerning animal welfare and the varying interpretations of the effects of confinement on laying hens and gestating sows are obvious examples of the consequences of disagreement on the concept of animal welfare.

While different concepts and consequently different methodologies may be used to assess animal welfare, 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 an intuitively aversive or rewarding condition reduces or improves animal welfare, respectively, on the basis of the measures of different concepts of animal welfare. The objective of the research conducted in this project was to examine the validity of the welfare criteria through the second approach: that is, test the hypothesis that restriction of a resource or behaviour that animals are highly motivated to access or perform, respectively, leads to disruption to biological function, such as occurrence of abnormal behaviour, increased stress and poor growth and health.

Previous research has shown that pigs vary in their motivation for social contact relative to feed in Y-maze preference tests. Therefore, using two highly preferred resources and resources for which individual pigs may differ in their preference, provides an excellent model to study the effects of restriction of preferred resources on biological function. Consequently, the approach was taken in this project to test the hypothesis that deprivation of social contact in pigs that highly prefer social contact results in disruption to biological function.

This experiment examined the effects of social restriction of pigs that were classified as strongly socially preferred or moderately socially preferred on disruption in biological function. It was found that when pigs were socially restricted by limiting their tactile contact or removing their visual and tactile contact with neighbouring pigs, the preference classification of the pig affected saliva cortisol concentrations: strongly socially preferred pigs had higher saliva cortisol concentrations.

This finding suggests that strongly socially preferred pigs are so highly motivated to have full tactile contact with pigs experience stress when restricted of tactile contact with pigs. An alternative but less likely explanation is that housing these pigs in individual pens either with limited tactile contact or no visual and tactile contact with neighbouring pigs leads to unresolved aggression in unsuccessfully attempting to establish a dominance relationship.

Surprisingly, while are numerous examples in the literature in both pigs and other species where housing, handling or husbandry effects that have affected cortisol concentrations have also affected behaviour, growth or reproduction, there was no evidence in the present experiment that preference classification affected behaviour or live weight gain.

Nevertheless, these results together with limited evidence from several other studies indicate that restriction of a resource that animals are highly motivated to access leads to disruption to biological function, such as occurrence of abnormal behaviour, increased stress and poor growth and health. These results should provide confidence to both scientists studying impacts on animal welfare and policy makers utilize science to inform animal welfare standards and recommendations that approaches examining biological function and animal preferences, providing the methodology is sound, provides the best current assessment of animal welfare. However, where the opportunity exists, any argument for impaired welfare due to restriction of a resource or behaviour would be strengthened by both evidence that animals are highly motivated to access the resource or perform the behaviour, respectively, and evidence of disruption to biological function.

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11. Publications

There are no current publications arising from this project, however, the research described in this report is in the process of being prepared for publication. Once the publication has been internally reviewed and deemed ready for publication it will be submitted to APL as per the requirements for requesting disclosure.