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# Relationships between Biological Function and Animal Preferences in Studying Animal Welfare

**Final Report**
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**The University of Melbourne**
**Animal Welfare Science Centre**
Prof Paul Hemsworth

Parkville Vic 3010

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**Section**

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## Non-Technical Summary Report

Principal Investigator: Professor Paul Hemsworth, Animal Welfare Science Centre, the University of Melbourne, Parkville, 3010, Victoria, Australia

Other Investigators: The late Dr. John Barnett, Guille Karlen and Bronwyn Stevens, Animal Welfare Science Centre, the University of Melbourne.

### *Aims of the Research*

The main aim of this research was to compare the two main approaches to welfare assessment, measuring biological functioning and animal preferences, by examining whether the resources that are the most preferred by animals are the same resources that animals, when deprived of them, show the most extreme coping attempts. A sound understanding of these two methodologies or measurements is important in the validation of welfare research methodology to establish welfare standards and develop tools to measure welfare in the field.

### *Rationale for the Research*

A current weakness in assessing animal welfare and subsequently in establishing welfare standards for animals is that there are differing definitions 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 preference tests, aversion learning and behavioural demand tests 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. There is also some emphasis on behavioural indicators of poor coping such as fearfulness, aggression and stereotypes, but these are generally indicators used in the above two approaches.

This scientific uncertainty in relation to animal welfare concepts raises 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, can these resultant methodologies be used to identify those conditions that lead to negative affective states. In other words, are those resources (or behaviours) where deprivation results in biological dysfunction on the basis of a broad examination of the behavioural, physiological, health and fitness responses of animals, the same resources (or behaviours) that animals highly prefer on the basis of preference tests? The objective of the research conducted in this project was to examine these questions.

Therefore, the general objective of this project was to improve our understanding of the relationship between these two main methodologies of animal welfare by testing the hypothesis that deprivation of highly preferred resources results in biological dysfunction. This fundamental research may assist in reducing the interpretative differences in animal welfare science.

***Brief Methodology***

To examine the relationships between two concepts of animal welfare, the biological functioning and feelings-based concepts, this project tested 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 pig preference was assessed by offering animals a choice of resources in a series of Y maze trials. The resources used were: social contact vs. feed (Experiment 1) and social contact vs. environmental enrichment (Experiment 2).

The approach taken to test this hypothesis was the use of a design in which pigs of two preference types were studied when deprived of either the more preferred or less preferred resource. Evidence supporting the hypothesis would be significant interactions between the main effects (i.e. preference type and form of preference) on the measures of biological functioning. For example, deprivation of the pig's more preferred resource creates biological dysfunction while deprivation of the less preferred resource does not result in biological dysfunction.

***Major Research Findings***

As expected in Experiment 1 based on previous research by the group, pigs differed in their preferences for feed and social contact. In Experiment 1, two pertinent findings 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: pigs in the so-called "Feed preferred" group weighed less than pigs in the so-called

“Social preferred group” when both groups were deprived of feed, while Social 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. While only 16 pigs were studied and the interaction on free cortisol concentration was a tendency only, this experiment provides limited evidence that that deprivation of a highly preferred resource may result in biological dysfunction.

Unexpectedly, pigs showed a very low preference for environmental enrichment toys in the Experiment 2. Pigs selected for study in Part 2 as so-called “Environmental enrichment preferred” group chose the environmental enrichment toys on average in only 14% of Y maze trials in Part 1. Consequently this experiment did not provide the desired model to study the effects of restriction of preferred resources on biological function: that is, pigs of two preference types studied when deprived of either the more preferred or less preferred resource.

***Conclusions***

The results of Experiment 1 provide limited evidence that deprivation of a highly preferred resource may result in biological dysfunction on the basis of adverse effects on stress physiology and productivity. However, this hypothesis requires more extensive testing than research to date.

It is recommended that further fundamental research is required to examine whether with these two methodologies can be integrated, thus developing a broader consensus on animal welfare methodologies. Such an outcome 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.

In the meantime, until the relationships between these two concepts of animal welfare are established it would be prudent to utilise both methodologies in studying conditions that may affect animal welfare. While animal preference studies provide evidence of the motivation of animals to access resources (or perform behaviours), additional evidence, particularly on occurrence of abnormal behaviour, stress physiology and health, are necessary to provide a confident assessment of the impact of restricting these resources (or behaviours) on the animal’s welfare. The opposite would also be prudent: assessments of biological functioning would be complemented by animal preference tests. However it is recognised that these two methodologies are costly in terms of animals, facilities and time and thus it is desirable to develop a broader consensus on animal welfare methodologies so that either approach can be used, particularly in situations in which one is easier or more practical to use than the other.

## Background to the Research

Animal welfare is an increasingly contributing perspective in society, strongly influencing the acceptability of various farm animal management options. 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 critical 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 considerable 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 feelings-based 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 'feelings' or subjective affective states to motivate behaviour to meet these needs. 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. In the early literature, the view that animals should perform their full 'repertoire' of behaviour was very common. 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 one of the most obvious and challenging limitations 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 contentious 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. 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, can we use these resultant methodologies identify those conditions that lead to negative affective states. In other words, are those resources (or behaviours) where deprivation results in biological dysfunction on the basis of a broad examination of the behavioural, physiological, health and fitness responses of animals, the same resources (or behaviours) that animals highly prefer on the basis of preference tests? The objective of the research conducted in this project was to examine these questions.

The rationale for the approach taken in this project to study this question is as follows. Food is considered as the "gold standard" in preference testing. Food is a fundamental biological requirement for animals and thus food is expected to be highly preferred, especially when animals are deprived. Matthews and Ladewig (1994) used operant testing to determine the amount of effort that 12-week-old male castrated pigs would work to obtain access to feed (27 g of pellets). The operant response was pressing on a nose plate to receive access to the resource. The researchers reported that the demand observed for food was almost completely inelastic, that is food continued to be chosen as the effort required to obtain it increased. However, it was found that demand observed for social contact was more elastic in that the choice for social contact declined as the effort required to obtain it increased. Nevertheless, previous research by the researchers (Hemsworth et al., unpublished data) using Y maze tests demonstrated differences between individual pigs in their preferences for feed and social contact. 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. Therefore the approach taken in Experiment 1 of this project was test the hypothesis that deprivation of the more preferred resource results in biological dysfunction by examining interactions between the main effects of preference type (either feed or social contact) and deprivation of resources (either feed or social contact). Evidence to support the hypothesis would be interaction between main effects on biological dysfunction. Therefore in this project a range of behavioural, physiological and fitness responses were used to assess biological functioning, while pig preference was assessed by offering animals a choice of two different resources in a series of Y maze trials in each experiment.

While somewhat controversial, particularly in relation to loose use of the term, environmental enrichment is considered important for animal welfare (Young 2003,). Therefore, while little is known of the preference of animals for environmental environment in preference tests, the two resources studied in Experiment 2 were environmental enrichment toys (an empty milk carton, a tennis ball a rope and a length of chain suspended across the pen by a rope) and social contact. As in Experiment 1, the approach taken in Experiment 2 was test the hypothesis that deprivation of the more preferred resource results in biological dysfunction by examining interactions between the main effects of preference type (in this case, either environmental enrichment toys or social contact) and deprivation of resources (either environmental enrichment toys or social contact). Evidence to support the hypothesis would be interaction between main effects on biological dysfunction. As in Experiment 1, a range of behavioural, physiological and fitness responses were used to assess biological functioning, while pig preference was assessed by offering animals a choice of two different resources in a series of Y maze trials in each experiment.

Thus two experiments were conducted in this project utilising this approach to examine whether depriving animals of their more preferred resource results in biological dysfunction. That is, deprivation of the pig's more preferred resource creates biological dysfunction while deprivation of the less preferred resource does not result in biological dysfunction.

## Objectives of the Research Project

The specific objective of this project was to compare the two main approaches to assess welfare: "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 on animal welfare methodologies. 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.

## Research Methodology, Detailed Results & Discussion of Results

### *Experiment 1*

*Aims*

This experiment aimed to test the hypothesis that deprivation of a highly preferred resource results in biological dysfunction. The two resources used in this experiment were social contact and feed.

*Materials and Methods*

The experiment consisted of two parts, the first part examined pig preferences for feed and social contact when simultaneously deprived of these two resources and the second part examined the biological functioning of pigs when deprived of the individual pig's more preferred resource, feed or social contact.

*Part 1*

The choice behaviour of 36 growing pigs for feed or social contact was studied in a series of Y maze trials over 12 days while the pigs were restricted of both feed and social contact. The pigs were female pre-pubertal Large White × Landrace crossbreds and at the start of the experiment were aged 10-14 weeks with an average weight of 39.95 kg. All pigs were reared in groups on concrete floors with no access to bedding and were provided *ad libitum* access to water and a commercial pelleted diet, which satisfied all metabolic requirements. During the 3-week experiment, the pigs were individually housed in 18 pens located in 3 rooms and each pen had a concrete floor with a floor space of 1.6 $m^2$. This study was conducted at the Victorian Department of Primary Industries research site in Werribee, Victoria and commenced in early autumn. The pigs were housed and tested in a steel-sided, mechanically ventilated building providing natural and artificial light, where artificial lighting, for approximately 9 h per day, illuminated 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 facilities. Pigs individually entered the start-box (2.0 x 1.5 m, length x width), where the pigs had visual contact with the two short arms of the Y maze (each 2.0 × 1.5 m) through a mesh gate that opened into the long arm of the maze (3.0 ×1.5 m). See Figure 1. The pigs in the start-box could see the choice options located at the ends of the short arms (feed in a feed trough ('feed' resource) in one arm and companion pigs ('social contact' resource) in the other arm). Solid-walled gates were located on the entry to each short arm so that once a pig entered a short arm of the Y maze, a gate on the entrance to the other arm was closed, precluding entry to this other arm for the remainder of the trial. Pigs were held in the start box for 5 s before testing and were allowed 2 min in one of the two short arms.

Training Phase - Week 1

During the first week of the experiment, pigs were individually housed in pens with visual and tactile contact with at least one neighbouring pigs through wire-mesh pen divisions. Training, which was conducted in the morning at least 30 minutes after feeding, involved introducing the pigs in pairs for 5 min to the empty maze in 2 sessions on day 1, followed by introduction in pairs into each arm of the maze (while the other arm was closed) for 2 min in 4 sessions over 2 days where the arms contained the two resources. This sequence of introduction into each arm of the maze containing one resource for 2 min was repeated in 6 sessions over 3 days, but the pigs were introduced individually. For training in pairs on days 1-3, pigs were randomly assigned to pairs and the pairing of the maze arm and the resource was randomly assigned to each pair of pigs. Furthermore, the pairing of arm to resource remained consistent for each pig in all training and testing trials. On days 2-6, the order of exposure of pairs of pigs (days 2-3) and subsequently individual pigs (days 4-6) to each arm was randomly assigned and each pair of pigs (days 2-3) and each pig (days 4-6) were daily introduced to the start box for 5 s and then allowed access to one of the short arms of the Y maze for a period of 2 min, before being returned to the start box and allowed access to the other short arm for 2 minutes.

During the training phase, pigs were fed daily (0900 h) in feed troughs in their individual pens by allowing them 30 min to freely consume a commercial pelleted diet, which satisfied all metabolic requirements. The average feed consumed by pigs in this training phase was used to estimate voluntary feed intake (VFI) in single daily feeding bouts for the study group of pigs.

Treatment and Testing Phase – Weeks 2 and 3

During this 2-week period pigs were daily (0900 h) fed 70% of their estimated VFI in feeding troughs in their individual pens and were housed individually with no tactile and visual contact with neighbouring pigs. Choice behaviour for feed or social contact was studied in daily trials over the first 12 days. All pigs were tested within 30 to 90 minutes of feeding to minimise differences in hunger between pigs within treatments.

Order of testing pigs was randomly generated for each day of testing. Both maze arms were available on every trial and so pigs were able to move into either arm in the choice area. Identical maze arm and resource pairings were maintained for each pig consistent with those used in the training phase. The procedure for each trial was similar to that described for the training phase. After 5 s in the starting-box, the pig was allowed into the long arm of the maze to enter one of the two short arms of the maze. Once the pig had fully entered one arm, the solid gate leading to the other arm was closed to prevent entry and sight of the alternative resource. After 2 min had elapsed, the pig was removed from the Y maze and returned to its pen.

The resource arm chosen, the time taken to approach within 5 cm of the resource and the time spent within 5 cm of the resource were recorded for each trial.

*Part 2*

From the 36 pigs studied in the first part of the experiment, 16 pigs were selected on the basis of their preferences:

1. "Feed preferred" pigs - 8 pigs that chose feed in the majority of Y maze trials.
2. "Social preferred" pigs - 8 pigs that chose social contact in the majority of Y maze trials.

After completing Part 1, these 16 pigs were housed in groups and were provided *ad libitum* access to water and a commercial pelleted diet, which satisfied all metabolic requirements. At an average age of 26 wks, pigs were individually housed for 6 weeks in 2.4 $m^2$ pens with partially slatted concrete floors, and during this period half of each of the two preference groups of pigs (i.e. 4 feed preferred and 4 social preferred pigs) were housed in one of the two following restrictions:

1. Feed restriction only – 70% of estimated VFI but provided with tactile and visual contact with one neighbouring pig through a wire-mesh pen divisions.
2. Social restriction only – individually housed with restricted social contact through the elimination of both visual and tactile contact with pigs but not olfactory and auditory contact with pigs. The pigs were fed 100% of estimated VFI.

*Behaviour*

Cameras were mounted over the pens to record the behaviour of the pigs on day 1 (0700-1900 h) and day 2 (0800-1400 h and 1600-1900 h) in week 4. From the video records, instantaneous sampling at 15-min intervals was used to record whether or not each of the following postures, lying, standing, walking, sitting and kneeling, and behaviours, interactions with floor, pen, neighbouring pig, feeder and drinker and idle, were shown by each pig.

*Live Weight*

Pigs were weighed at an average age of 30 wks and then 3 weeks later.

*Stress Physiology*

After 5 weeks of treatment, the pigs were catheterised under general anaesthesia and 6 days later serial blood samples (5 mL) were collected via the catheter, using Monovettes®

(Sarstedt Australia, SA) coated with lithium heparin, at 1-h intervals between 0800 and 1700 h, to measure the day-time profile in cortisol concentrations of the pigs. Single intra-muscular injection of adrenocorticotropic hormone (ACTH; 50 IU Synacthen, Ciba Geigy, Lane Cove, NSW) and a single dose of intra-venous corticotrophin releasing hormone (CRH, 8.7 pmol/kg body weight of synthetic CRH (Sigma Pharmaceuticals, Missouri) in physiological saline) were administered to the pigs at 1245h at 7 and 8 days post-surgery, respectively, and blood samples were collected (as described above) 15minutes prior to each of the injections and then at intervals 10, 15, 30, 45, 60, 90, 120, 180 and 240 minutes post-injection. The rationale for the 'ACTH' and 'CRH' tests is that chronic stress generally results in higher cortisol responses to exogenous ACTH and CRH (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 and CRH responses and 'day-time profile') and free cortisol ('day-time profile') concentrations.

Plasma concentrations of total cortisol were determined in duplicate 100-μL aliquots using an extracted radioimmunoassay according to the protocol developed by Bocking and Harding (1986) and validated for pig plasma using hydrocortisone H-4001 (Sigma Chemical Co., St Louis, MO) as standard. Free cortisol concentrations were determined using an ultrafiltration/ligand binding method as described in Ho *et al*. (2006). The sensitivity for assays ranged from 0.44 to 0.49 ng/ml.

*Statistics*

To test the hypothesis that deprivation of a preferred resource results in biological dysfunction, the interactions between main effects on behaviour, stress physiology and live weight were examined in Part 2. Univariate General Linear Model (SPSS 16.0, SPSS Inc., Chicago, Illinois, USA) was used to examine the two main effects, preference (feed or social) and restriction (feed or social), in this experiment on pig behaviour, physiology and live weight.

The distribution of choice behaviour in Part 1 was examined by conducting a hierarchical analysis using a dendrogram algorithm (SPSS 16.0, SPSS Inc., Chicago, Illinois, USA). Behaviour data in Part 2 are presented as the frequency of observation sessions in which the individual posture or behaviour was observed and are referred to as the frequency of the posture or behaviour. The cortisol values of the 10 samples collected hourly from each pig from 0800 and 1700 h were averaged for each pig to provide an estimate of the day-time cortisol concentrations. The highest cortisol concentrations from the 9 samples collected from each pig following the ACTH and CRH injections were identified as the maximum cortisol response to ACTH and CRH, respectively.

A number of measurements were transformed prior to the analysis of variance to avoid the residual variation increasing as the mean increased.

*Results*

Part 1

The distribution of feed choice of the 36 pigs in the daily Y-maze trials over 12 days is shown in Figure 2. A hierarchical analysis using a dendrogram algorithm of this distribution of choice behaviour indicated that there were two main clusters of pigs, with the separation of clusters between 33% and 50% of trials in which feed was chosen. That is, the first cluster was comprised of 14 pigs (39% of pigs) which chose feed in the Y maze in 0 to 33% of the trials and the second cluster, consisting of 22 pigs (61% of pigs), which chose feed in 50 to 100% of trials.

Part 2

From the 36 pigs studied in the first part of Experiment 1, 8 pigs that chose feed in the majority of Y maze trials (average choice of feed in 80% of trials, labelled "feed preferred" group of pigs) and 8 pigs that chose social contact in the majority of Y maze trials (average choice of social contact in 91% of trials, labelled "social preferred" group of pigs) were selected for study in Part 2.

Behaviour

There were significant ($P<0.05$) main effects on the frequency of tactile interactions with pigs (Table 1). Not surprisingly, pigs that were deprived of tactile and visual contact with pigs had less tactile interactions with pigs than those that were deprived of feed. Furthermore, pigs that preferred social contact in the Y maze trials in Part 1 interacted more ($P<0.05$) with neighbours that those that preferred feed in the Y maze trials. However, there was a significant ($P=0.035$) interaction effect on tactile interactions with neighbours: social preferred pigs had a higher frequency of tactile interactions with pigs when feed deprived (back transformed mean of 1.75 interactions observed over 25 h) than feed preferred pigs when feed deprived (0.50 interactions).

There were no other significant ($P=0.05$) main effects on the frequency of the main postures and other behaviours studied (Table 1). There was a tendency ($P=0.067$) for an effect of restriction on the frequency of tactile interactions with the feeder (Table 1): social restriction tended to increase the frequency of interactions with the feeder in comparison to feed restriction. There were no significant ($P=0.05$) interactions on the frequency of the main postures and other behaviours

Live Weight

There were no significant (P=0.05) main effects on the final live weight of pigs at the end of treatment in Part 2 (Table 1). However, there was a significant (P=0.030) interaction on live weight. As shown in Table 2, when deprived of feed, the feed preferred pigs weighed less at the end of Part 2 than social preferred pigs. In contrast, when deprived of social contact, the socially preferred pigs weighed less than the feed preferred pigs.

Stress Physiology

There were main effects on cortisol concentrations (Table 3). While there was no significant (P=0.05) effects of preference, deprivation affected the day-time average total cortisiol concentrations: feed restriction increased (P=0.044) total cortisol concentrations compared to social restriction. There was a similar trend for free cortisiol concentrations (P=0.079). Interestingly, there was a tendency (P=0.110) for an interaction between main effects on free cortisol concentrations (Table 2); when deprived of feed, the feed preferred pigs tended to have higher cortisol concentrations than social preferred pigs and in contrast, when deprived of social contact, the social preferred pigs tended to have marginally higher cortisol concentrations than the feed preferred pigs.

Deprivation affected the peak total cortisol concentration to CRH (Table 3): peak cortisol concentrations were higher (P=0.013) in feed deprived than socially deprived pigs. There was also a tendency (P=0.072) for preference to affect the peak total cortisol concentration to ACTH (Table 3), with higher concentrations in the feed preferred pigs. There were no interaction effects on day-time average total cortisol concentration (P = 0.217), peak total cortisol concentration to ACTH (P=0.637) and peak total cortisol concentration to CRH (P=0.288).

*Discussion*

As seen in previous research (Hemsworth et al., unpublished data), there were two main clusters of pigs, one that preferred feed and one that preferred social contact in the Y maze trials. The separation of the clusters in the present experiment was between 33% and 50% of trials in which feed was chosen. The first cluster was comprised of 14 pigs (39% of pigs) which chose feed in the Y-maze in 0 to 33% of the trials and the second cluster, consisting of 22 pigs (61% of pigs), which chose feed in 50 to 100% of trials. The proportion of pigs in the two clusters found in the present experiment is different from our previous research (Hemsworth et al., unpublished data). In the previous experiment, the first cluster ('social preferred') comprised of 36 pigs (75% of pigs) which chose feed in the Y-maze in 0 to 50% of the trials and the second cluster ('feed preferred') compromised of 12 pigs (25% pigs), which chose feed in 75 to 91% of trials. There were several differences in the two studies. In Hemsworth et al. (unpublished data), the pigs were aged 15-20 weeks at the start of the study with an average weight of 31.5 kg while in the current study, pigs were 10-14 weeks with an average weight of 40.0 kg. Preference testing in the present study commenced in early autumn in the present experiment, while testing in the study by Hemsworth et al. (unpublished data), which was conducted in two parts one in Australia and one in the USA, was conducted in mid summer and late spring, respectively. This age and seasonal differences between studies may have contributed to study differences in motivation to access feed or social contact in the y maze trials. However, while the proportion of pigs in these two clusters differs between studies, both studies suggest that there may be two types of pigs in terms of their choice behaviour. If this is a real effect, these results have important implications for animal welfare. One interpretation for example is that pigs may differ in their long-term choice behaviour for feed or social contact and thus perhaps their welfare requirements in relation to these two resources. If this is a real effect, it indicates the complexity of welfare requirements in relation to resources in general. Furthermore, these results raise some fascinating questions about the genetic and/or experiential basis for these two apparent types of pigs.

The main aim of this experiment 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 social 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 social preferred pigs tended to have higher cortisol concentrations than the feed preferred pigs when deprived of social contact.

While little is known of these two types of pigs, the feed preferred pigs may have a higher metabolic requirement and thus voluntary feed intake than the social preferred pigs. Therefore, feed deprivation may result in a greater deficit in meeting their metabolic requirements than feed deprivation for the social preferred pigs and this is supported by the lower final live weight of the feed preferred pigs than the social preferred pigs. Activation of the hypothalamic-pituitary-adrenal (HPA) axis and the associated catabolic effects of ACTH and corticosteroids may assist in meeting a deficit in the animal's metabolic requirements and this is a possible explanation for the tendency for higher free cortisol concentrations and the lower live weights of the feed preferred than social preferred pigs when deprived of feed. In relation to social deprivation, this restriction may be more stressful for the socially preferred pigs than the feed preferred pigs and result in activation of the HPA axis and growth inefficiencies in the former group of pigs. Indeed there is some evidence for this. In the experiment by Hemsworth et al. (unpublished data) in which 75% of the study pigs preferred social contact over feed, social deprivation reduced growth rate. While there was no evidence that social deprivation in this previous research suppressed feed intake, the authors concluded that a likely interpretation of the reduced ADG 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 ADG.

These effects on live weight and free cortisol concentration provide limited evidence that deprivation of a highly preferred resource may result in biological dysfunction, in this situation, an inefficiency in growth. However, there were no main effect interactions on day-time total cortisol concentrations or the peak cortisol response to CRH and ACTH. It is likely that a change in cortisol dynamics that increases free cortisol concentrations is associated with a reduction in growth rate. Free cortisol is the biologically active hormone fraction and is a major factor involved in the process of gluconeogenesis, the conversion of non-carbohydrate sources ie. body protein, into glucose as an energy source to meet metabolic requirements. The consequence of this tissue mobilization, in the situation of the current experiment where *ad libitum* feed was not available, is a reduction in body weight. Furthermore, prolonged activation of the HPA axis with prolonged secretion of free cortisol leads to suppression of GH secretion, while free cortisol can induce resistance in target tissues to the effects of GH, insulin-like growth hormone factor I and other growth factors, and consequently the suppression of growth (Kaltas and Chrousos, 2007).

There were no main effects on the frequency of the main postures and behaviours of the pigs. The only exception was a significant interaction effect on social interactions: social preferred pigs had a higher frequency of tactile interactions with the neighbouring pig when feed deprived than feed preferred pigs when feed deprived. This finding is not unexpected since when pigs were feed deprived, they had opportunity to interact with their neighbour and the socially preferred pigs were expected to interact more since these pigs were classified as social preferred on the basis of their choice behaviour in the earlier Y maze trials. The tendency observed for social restriction to increase the frequency of interactions with the feeder in comparison to feed restriction may be a redirected behaviour arising from social restriction, although such behaviour was not seen with feed deprivation.

There were main effects on stress physiology. In comparison to socially deprived pigs, feed deprived pigs had higher day-time average total cortisiol concentrations, a tendency for higher day-time average free cortisiol concentrations and a higher peak cortisol concentration to CRH. This increase in cortisol concentrations in the latter pigs is likely to be a consequence of gluconeogenesis, the conversion of non-carbohydrate sources, that is, body protein into glucose as an energy source to meet metabolic requirements.

Therefore, this experiment provides limited evidence that 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. Only 16 pigs were studied and the interaction on free cortisol concentration was a tendency only, nevertheless, further research is warranted to address this effect.

***Experiment 2***

*Aims*

This experiment continues from Experiment 1 testing the hypothesis that deprivation of a highly preferred resource results in biological dysfunction. While the two resources studied in the first experiment were social contact and feed, social contact and environmental enrichment toys were studied in Experiment 2.

*Materials and Methods*

The approach in this experiment was similar to that used in Experiment 1 in that the first part of the experiment examined pig preferences for the two resources when simultaneously deprived of these two resources and the second part examined the effects of deprivation of the resources on the biological functioning of pigs that differed in their preferences. In contrast to the previous experiment when preferences were only studied when the pigs were deprived of the resources under study, in the present experiment preferences for the two resources was initially studied for 1 week when restricted and then studied for 1 week when restricted.

*Part 1*

In the first part of the experiment, the choice behaviour of 36 pigs (over two time replicates of 18 pigs) for environmental enrichment or social contact was studied over 14 days in Y maze trials. This experiment used the same custom built Y maze apparatus as in Experiment 1 (see Figure 1). The pigs were female pre-pubertal Large White × Landrace crossbreds and at the start of the experiment were aged 6-8 weeks with an average weight of 19.16kg. All pigs were reared in groups on concrete floors with no access to bedding and were provided *ad libitum* access to water and a commercial pelleted diet, which satisfied all metabolic requirements. During the 3-week experiment for each replicate, the pigs were individually housed in 18 pens located in 3 rooms and each pen had a concrete floor with a floor space of 1.6 $m^2$. This study was conducted at the Department of Primary Industries Victoria research site at Werribee, Victoria.

During the study, the pigs were fed *ad lib*, and water drinkers were available at the front of each pen.

Training Phase - Week 1

During the first week of the experiment, pigs were individually housed in pens with visual and tactile contact with at least one neighbouring pigs through wire-mesh pen divisions and provided with environmental enrichment in the form of a ball and empty milk bottle suspended by a chain.

Training, which was conducted in the morning at least 30 minutes after feeding, involved introducing the pigs in neighbouring groups of three for 5 min to the empty maze in 2 sessions on day 1, followed by introduction in groups of three into each arm of the maze (while the other arm was closed) for 2 min in 4 sessions over 2 days where the arms each contained one of the two resources under study (social contact or environmental enrichment). A ball and empty milk bottle was suspended by a chain ('environmental enrichment') in one of the short arms of the y maze while companion pigs ('social contact') were allocated to the adjacent pen behind the other short arm. The pairing of maze arm and resource was randomly pseudo-assigned to each group of three pigs (in both the training and testing trials) so that each resource was equally represented in each arm in each trial and the location of resources remained consistent for each pig in both the training and testing trials.

This sequence of introduction into each arm of the maze containing one resource for 2 min was repeated for another 2 days, but the pigs were introduced individually. Each pig was kept in the starting box for 5-10 seconds, and then released into the maze with one arm opened at a time and allowed to interact with one resource for about 2 min. The pig was then returned to the start box and the procedure was repeated with the other resource.

During the training phase, pigs were fed daily (0900 h) in feed troughs in their individual pens.

Treatment and Testing Phase – Weeks 2 and 3

During the first week of testing (i.e. Week 2), pigs were housed with unrestricted access to the environmental enrichment toys and social contact as described above during familiarization and training. During the second week (Week 3), the pigs were socially restricted from their neighbours by covering up the wire mesh divisions with a large piece of black ply wood. The environmental enrichment toys were also removed from the pens.

Order of testing pigs was randomly generated for each day of testing. Both maze arms were available on every trial and so pigs were able to move into either arm in the choice area. Identical maze arm and resource pairings were maintained for each pig consistent with those used in the training phase. The procedure for each trial was similar to that described for the training phase. After 5 s in the starting-box, the pig was allowed into the long arm of the maze to enter one of the two short arms of the maze. Once the pig had fully entered one arm, the solid gate leading to the other arm was closed to prevent entry and sight of the alternative resource. After 2 min had elapsed, the pig was removed from the Y maze and returned to its pen.

The resource arm chosen, the time taken to approach within 5 cm of the resource and the time spent within 5 cm of the resource were recorded for each trial. A video camera mounted on the ceiling above the Y maze was used to record the behaviour of the pigs whilst in the Y maze. Live weights for each pig were also recorded.

At the end of testing of each replicate (ie the 2-week testing period), the pigs were transferred to an alternate location and again individually housed in pens with a floor space of 3.2 $m^2$. The pigs had visual and tactile contact with at least one neighbouring pig through wire-mesh pen divisions and were provided with the environmental enrichment toy in the pen.

*Part 2*

Pigs in Part 1 showed an overwhelming preference for social contact in the Y maze (average choice of social contact was 94% of trials, with choice behaviour of individual pigs for social contact ranging from 43% to 100% of trials). Therefore, in order to study pigs in which choice behaviour differed as much as possible, two groups from the 36 pigs studied in the first part of Experiment 2 were selected for study in Part 2, 12 pigs that chose social contact in 100% of the Y maze trials (labelled "social preferred" group of pigs) and 12 pigs that chose social contact in the least number of Y maze trials (labelled for the purpose of this report "environmental enrichment preferred" group of pigs).

Half of each of these two groups (i.e. 6 social preferred and 6 environmental enrichment preferred pigs) was housed in one of the two following restrictions for 6 weeks:

1. Environmental enrichment restriction only – No environmental enrichment toys in pen (individually housed but with tactile and visual contact with neighbouring pigs through wire-mesh pen divisions).
2. Social restriction only – individually housed with tactile and visual restriction with pigs (but access to environmental enrichment toys (a ball and empty milk bottle suspended by a chain) in pen).

All pigs were fed 3kg of commercial diet daily at 8am. The effects of these restrictions on the behaviour and stress physiology of these two types of pigs were studied.

*Live Weight*

Pigs were weighed at the beginning of the treatment period (week 1, part 2) and then 6 weeks later.

*Behaviour*

Cameras were mounted over the pens to record the behaviour of the pigs over two days (0700-1900 h) in week 4 of treatment. From the video records, instantaneous sampling at 15-min intervals was used to record whether or not each of the following postures, lying, standing, walking, sitting and kneeling, and behaviours, interactions with floor, pen, neighbouring pig, toy, feeder and drinker and idle, were shown by each pig.

*Stress Physiology*

In week 5 of treatment indwelling catheters were surgically implanted into the vena cava via the cephalic vein under full anaesthesia. Each pig was treated intramuscularly with Trivetrin antibiotic (1mL/15kg body weight) during surgery, and analgesia in the form of Flunixin (1mL/30kg) was intramuscularly administered post surgery. The pigs were given a minimum of four days post operative care to recover from surgery.

As in experiment 1, serial blood samples (9 mL) were collected in week 6 of treatment (day 4, post surgery) via the catheter, using Monovettes® (Sarstedt Australia, SA) coated with lithium heparin, at 1-h intervals between 0800 and 1700 h, to measure the day-time profile in cortisol concentrations of the pigs. An additional sample was collected at 0900 on this day in an EDTA coated Monovette® and sent to a commercial laboratory for a white blood cell differential. A single intra-muscular injection of adrenocorticotropic hormone (ACTH; 50 IU Synacthen, Ciba Geigy, Lane Cove, NSW) and a single dose of intra-venous corticotrophin releasing hormone (CRH, 8.7 pmol/kg body weight of synthetic CRH (Sigma Pharmaceuticals, Missouri) in physiological saline) were administered to the pigs at 1245h at 7 and 8 days post-surgery, respectively, and blood samples were collected (as described above) 15minutes prior to each of the injections and then at intervals 10, 15, 30, 45, 60, 90, 120, 180 and 240 minutes post-injection. The blood samples were centrifuged and the plasma was stored frozen at –18 °C until assayed for total cortisol (ACTH and CRH responses and ‘day-time profile’), free cortisol (‘day-time profile’) and ACTH (CRH responses) concentrations.

*Results*

Part 1

As reported above, the majority of pigs overwhelming choose social contact in the Y maze trials. During the unrestricted period of testing, the average choice of social contact was 94% of trials (with choice behaviour of individual pigs for social contact ranging from 43% to 100% of trials). In other words, environmental enrichment was only chosen on average in 6% of trials. Only one quarter of the pigs choose environmental enrichment at least once.

Part 2

The 12 pigs selected in Part 1 as the "environmental enrichment preferred" group of pigs (ie the 12 pigs that chose social contact in the least number of Y maze trials) chose social contact on average in 86% of trials while "social preferred" group of pigs chose social contact on average in 100% of trials.

There was some slight variation in choice behaviour of individual pigs in the unrestricted and restricted testing periods (Figs. 3 and 4) but the selection of pigs for use in Part 2 was based on their choice behaviour during the unrestricted testing period (as in Experiment 1).

Behaviour

There were significant ($P<0.05$) main effects on the frequency of tactile interactions with pigs (Table 4). Not surprisingly, pigs that were deprived of tactile and visual contact with pigs had less tactile interactions with pigs than those that were deprived of environmental enrichment. Similarly there were significant ($P<0.05$) main effects on the frequency of tactile interactions with the environmental enrichment toys with those deprived of environmental enrichment toys interacting less than those deprived of social contact. There were no significant main effects on any other behaviours. However there was a strong trend ($p=0.051$) towards an interaction between the main effects on the frequency of standing. The environmental enrichment preferred pigs that were deprived of environmental enrichment stood in a greater proportion of the observations than the social preferred pigs that were deprived of environmental enrichment. Similarly the social preferred pigs that were deprived of social contact stood in a greater proportion of the observations than the environmental enrichment preferred pigs that were deprived of social contact.

Live Weight

There were no significant ($P=0.05$) main effects on the final live weight of pigs at the end of treatment in Part 2 (Table 4). However, there was a significant ($P=0.047$) interaction on live weight. As shown in Table 5, when deprived of social contact, the social preferred pigs weighed more at the end of Part 2 than environmental enrichment preferred pigs. In contrast, when deprived of environmental enrichment, the environmental enrichment preferred pigs weighed more than the social preferred pigs.

Stress Physiology

There were no main effects or interactions between main effects on total or free cortisol concentrations (Table 6). Furthermore, there were no main effects or interactions between main effects on peak total cortisol in response to ACTH or CRH, or on peak ACTH response to CRH.

*Discussion*

Unexpectedly, pigs showed a very low preference for environmental enrichment toys in this experiment. Pigs selected for study in Part 2 as so-called "Environmental enrichment preferred" group chose the environmental enrichment toys on average in only 14% of trials in Part 1. Consequently this experiment did not provide the desired model to study the effects of restriction of preferred resources on biological function: that is, pigs of two preference types studied when deprived of either the more preferred or less preferred resource.

As discussed in more detail in the next section, it is difficult to conclude with any confidence that those pigs that chose social contact in all trials highly preferred social contact since the alternative (environmental enrichment toys) was clearly markedly less attractive Thus the results of Experiment 2 provide little evidence either way to support the hypothesis that deprivation of a highly preferred resource results in biological dysfunction. Nevertheless, the general findings are discussed here.

As reported above, the majority of pigs overwhelming choose social contact in the Y maze trials. The 12 pigs selected in Part 1 as the "Environmental enrichment preferred" group of pigs (i.e. the 12 pigs that chose social contact in the least number of Y maze trials) chose social contact in 86% of trials while "Social contact preferred" group of pigs chose social contact on average in 100% of trials.

There was a strong trend ($p=0.051$) towards an interaction between the main effects on the frequency of standing. The environmental enrichment preferred pigs that were deprived of environmental enrichment stood in a greater proportion of the observations than the social preferred pigs that were deprived of environmental enrichment. Similarly the social preferred pigs that were deprived of social contact stood in a greater proportion of the observations than the environmental enrichment preferred pigs that were deprived of social contact. Increased standing in pigs may be indicative of problems adapting with the deprivation. However, while this interpretation may provide some support for the project hypothesis, as suggested above, there are issues with the classification of the preference types in this experiment. Furthermore, there is no evidence of significant interactions between main effects on stress physiology that would support this interpretation of increased standing.

Surprisingly, there was a significant interaction on live weight. When deprived of social contact, the social preferred pigs weighed more at the end of Part 2 than environmental enrichment preferred pigs. In contrast, when deprived of environmental enrichment, the environmental enrichment preferred pigs weighed more than the social preferred pigs. There is no obvious explanation for this finding but again it should be recognised that there are issues with the classification of the preference types in this experiment and there is no evidence of significant interactions between main effects on stress physiology.

### ***General Discussion & Conclusions***

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. 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 general aim of this project was to develop a broader consensus on animal welfare methodologies which may assist in reducing the interpretative differences in animal welfare science. Improved understanding in this area is critical to the welfare debate and informing policy decisions that are acceptable to the community.

Understanding the relationship between the two main methodologies of animal welfare assessment, biological functioning and animal preferences, may lead to the development of a broader scientific consensus that these two methodologies can basically identify those conditions that lead to negative affective states. In other words, are those resources (or behaviours) where deprivation results in biological dysfunction on the basis of a broad examination of the behavioural, physiological, health and fitness responses of animals, the same resources (or behaviours) that animals highly prefer on the basis of preference tests?

Therefore the approach taken in Experiment 1 of this project was test the hypothesis that deprivation of the more preferred resource results in biological dysfunction by examining interactions between the main effects of preference type (either feed and social contact) and deprivation of resources (either feed or social contact). Evidence to support the hypothesis would be interaction between main effects on behaviour and physiological indicators of biological dysfunction, such as abnormal behaviour, stress and fitness effects.

It was found in Experiment 1 that there marked differences between individual pigs in their preferences for feed and social contact based on their choice behaviour in Y maze trials in which pigs had the choice of either resource. A similar finding had been shown in previous research by the researchers. It is known in general that pigs are highly motivated to access both feed and social contact and while pigs may differ in their relative preferences for these resources, the use of these two highly preferred resources provides an excellent model to study the effects of restriction of preferred resources on biological function. Experiment 1 tested the hypothesis that deprivation of the more preferred resource results in biological dysfunction by examining interactions between the main effects of preference type (either feed or social contact) and deprivation of resources (either feed or social contact). The findings of a significant interaction between the main effects of deprivation and preference on live weight and a tendency for an interaction between main effects of deprivation and preference on free cortisol concentrations indicate that deprivation of pigs of their more preferred resource (feed or social contact), but not their less preferred resource, resulted in lower live weights and higher cortisol concentrations. There were no interactions between main effects on behaviour of the pigs. While only 16 pigs were studied and the interaction on free cortisol concentrations was a tendency only, this experiment provides limited evidence that that deprivation of a highly preferred resource may result in biological dysfunction.

In contrast to Experiment 1 in which there were two clusters of pigs, one that predominantly preferred feed and one that predominantly preferred social contact in the Y maze trials, pigs in Part 1 in Experiment 2 overwhelming chose social contact, with a few pigs choosing environmental enrichment toys in a few trials. This creates a serious limitation in studying the hypothesis that deprivation of the highly preferred resources results in biological dysfunction since it is difficult to conclude with any confidence that those pigs that chose social contact in all trials highly preferred social contact. Those pigs that chose social contact in 100% of trials may not have had a high motivation for social contact since the alternative was clearly markedly less attractive on the basis that the average choice of the environmental enrichment toys was only 6% of trials (with choice behaviour of individual pigs for social contact ranging from 0% to 57% of trials). Furthermore, those pigs selected the "Environmental enrichment preferred" group chose the environmental enrichment toys on average in only 14% of trials.

Therefore, while it was not expected to find interactions between main effects on the biological variables studied in Part B of Experiment 2 because of these difficulties in classifying their preferences, the effects of social restriction on biological dysfunction may have been apparent if there was a high proportion of pigs that highly preferred social contact. However, there is no objective data to suggest that the pigs were consistently and highly motivated to access social contact. Thus the results of Experiment 2 provide little evidence either way to support the hypothesis.

In conclusion, Experiment 1 in this project provides limited evidence that 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. Only 16 pigs were studied and the interaction on free cortisol concentration was a tendency only, nevertheless, further research is warranted to address this effect.

## Implications & Recommendations

The scientific uncertainty in relation to animal welfare concepts does not necessarily diminish the robustness of the research utilising criteria or methodologies promulgated by the different concepts on animal welfare. However, it has several implications for identifying and resolving genuine risks to an animal's welfare.

First, 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 policymakers on the concept and definition of animal welfare can lead to disagreement on animal welfare-related policy and legislation. Although decisions on specific animal use are affected by a number of considerations, including scientific information of the harms and benefits to the animal, these differences in concepts, definitions, and (in turn) assessment lead to differences between policymakers in industry, community groups, and government in their interpretation of the validity of scientific information arising from a specific methodology. Consequently, these differences between policymakers in interpreting similar information can lead to disagreement on setting or accepting specific animal welfare standards. This is illustrated internationally in the variety of interpretations and standards relating to sow and laying hen housing.

Third, it is important in any welfare monitoring scheme in the field that the emphasis is on the animal and thus on those measures that best reflect lack of animal suffering: good health and lack of pain, injuries, and negative emotions. As outlined earlier in this report, others argue that positive emotions are equally important (Duncan, 2004). 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.

There are several commonalities in the rationale for the two main approaches of welfare assessment, biological functioning and animal preferences. For example, it is considered that animals, at least in the wild, will be motivated to choose those resources or behaviors that maintain homeostasis or biological functioning to optimize their fitness; that is, optimize their growth, reproduction, injury status, health, and survival. Furthermore, feelings or subjective affective states have evolved to motivate behavior 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; second, with the finding that an intuitively aversive condition reduces animal welfare on the basis of the measures of different concepts of animal welfare. Therefore, further research examining the validity of these concepts—and, in turn, methodologies—is necessary to understand the relationships between the concepts and indeed minimize these conceptual and methodological differences. The development of a broader scientific consensus on welfare measures arising from this research should lead to the development of credible measures that can be incorporated into welfare assessment and screening tools in the field.

The results of Experiment 1 tested the hypothesis that deprivation of the more preferred resource results in biological dysfunction by examining interactions between the main effects of preference type (either feed or social contact) and deprivation of resources (either feed or social contact). The findings of a significant interaction between the main effects of deprivation and preference on live weight and a tendency for an interaction between main effects of deprivation and preference on free cortisol concentrations indicate that deprivation of pigs of their more preferred resource (feed or social contact), but not their less preferred resource, resulted in lower live weights and higher cortisol concentrations. While only 16 pigs were studied and the interaction on free cortisol concentrations was a tendency, this experiment provides limited evidence that that deprivation of a highly preferred resource may result in biological dysfunction. In an experiment by Hemsworth et al. (unpublished data) in which 75% of the study pigs preferred social contact over feed, social deprivation reduced growth rate. While there was no evidence that social deprivation in this previous research suppressed feed intake, the authors concluded that a likely interpretation of the reduced growth rate in the socially deprived pigs was the catabolic effects of ACTH and corticosteroids: social deprivation, through the energetic cost of coping with a stressful situation, may have reduced growth.

It is therefore recommended that further fundamental research, with the overall objective of integrating these criteria and developing a broader consensus on animal welfare methodologies, should be undertaken. Another resource that may be highly attractive is human contact for pigs that have been positively handled. Variation in preference for human contact can be achieved through variation in a handling and thus using this resource and either feed or social contact, both of which are highly attractive but variable between pigs, provides another good model to study the effects of restriction of preferred resources on biological function. Pigs of two preference types can be studied when deprived of either the more preferred or less preferred resource, a design that was possible in Experiment 1 but not Experiment 2.

**Detailed Description of Intellectual Property**

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

**Technical Summary**

Scientists differ in their concept of animal welfare and thus their methodologies used to study animal welfare. Two main methodologies have been used to judge animal welfare: animal welfare: the welfare of animals has been assessed on the basis of either biological functioning or animal preferences. This uncertainty in relation to animal welfare methodologies does not necessarily diminish the robustness of the research utilising these criteria or methodologies, but it raises several concerns both within science and more broadly when decisions on acceptable welfare standards are being made by individuals or the community. Addressing this unease is fundamental to reducing the interpretative differences in animal welfare science.

The first approach in judging animal welfare consists of using a range of 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 approach uses preference tests, aversion learning and behavioural demand tests 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. There is some commonality in the rationale of these two approaches: the first utilises evidence of biological dysfunction to identify welfare problems while the second utilises evidence of animal preferences for conditions or behaviours and aversion of conditions or behaviours on the basis that these preferences should facilitate biological functioning. This commonality raises 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, can we use these resultant methodologies identify those conditions that lead to negative affective states. In other words, are those resources (or behaviours) where deprivation results in biological dysfunction on the basis of a broad examination of the behavioural, physiological, health and fitness responses of animals, the same resources (or behaviours) that animals highly prefer on the basis of preference tests? The objective of the research conducted in this project was to examine these questions.

Therefore, this research examined the hypothesis that deprivation of highly preferred resources results in biological dysfunction. A range of behavioural, physiological and fitness responses were used to assess biological functioning in this experiment, while pig preference was assessed by offering animals a choice of resources in a series of Y maze trials. The resources used were: social contact vs. feed (Experiment 1) and social contact vs. environmental enrichment (Experiment 2). The approach taken to test this hypothesis was the use of a design in which pigs of two preference types were studied when deprived of either the more preferred or less preferred resource. Evidence supporting the hypothesis would be significant interactions between the main effects (ie preference type and form of preference) on the measures of biological functioning. For example, deprivation of the pig's more preferred resource creates biological dysfunction while deprivation of the less preferred resource does not result in biological dysfunction.

As expected in Experiment 1 based on previous research by the group, pigs differed in their preferences for feed and social contact. In Experiment 1, two pertinent findings 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 P=0.030) between the main effects of deprivation and preference on live weight: pigs in the so-called "Feed preferred" group weighed less than pigs in the so-called "Social preferred group" when both groups were deprived of feed, while Social preferred pigs weighed less than Feed preferred pigs when both groups were socially deprived. There was also a tendency for an interaction (P=0.110) between main effects on day-time plasma 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. While only 16 pigs were studied and the interaction on free cortisol concentration was a tendency only, this experiment provides limited evidence that that deprivation of a highly preferred resource may result in biological dysfunction.

Unexpectedly, pigs showed a very low preference for environmental enrichment toys in the Experiment 2. Pigs selected for study in Part 2 as so-called "Environmental enrichment preferred" group chose the environmental enrichment toys on average in only 14% of Y maze trials in Part 1. Consequently this experiment did not provide the desired model to study the effects of restriction of preferred resources on biological function: that is, pigs of two preference types studied when deprived of either the more preferred or less preferred resource.

In conclusion, the results of Experiment 1 provide limited evidence that deprivation of a highly preferred resource may result in biological dysfunction on the basis of adverse effects on stress physiology and productivity. A previous experiment by the researchers also supports this finding. However, this hypothesis clearly requires more extensive testing than research to date. It is recommended that further fundamental research is required to examine whether these two methodologies can be integrated, thus developing a broader consensus on animal welfare methodologies. Such an outcome 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.

In the meantime, while animal preference studies provide evidence of the motivation of animals to access resources (or perform behaviours), additional evidence, particularly on occurrence of abnormal behaviour, stress physiology and health, are necessary to provide a confident assessment of the impact of restricting these resources (or behaviours) on the animal's welfare. The opposite would also be prudent: assessments of biological functioning would be complemented by animal preference tests. However it is recognised that these two methodologies are costly in terms of animals, facilities and time and thus it is desirable to develop a broader consensus on animal welfare methodologies so that either approach can be used, particularly in situations in which one is easier or more practical to use than the other.

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**Tables and Figures**

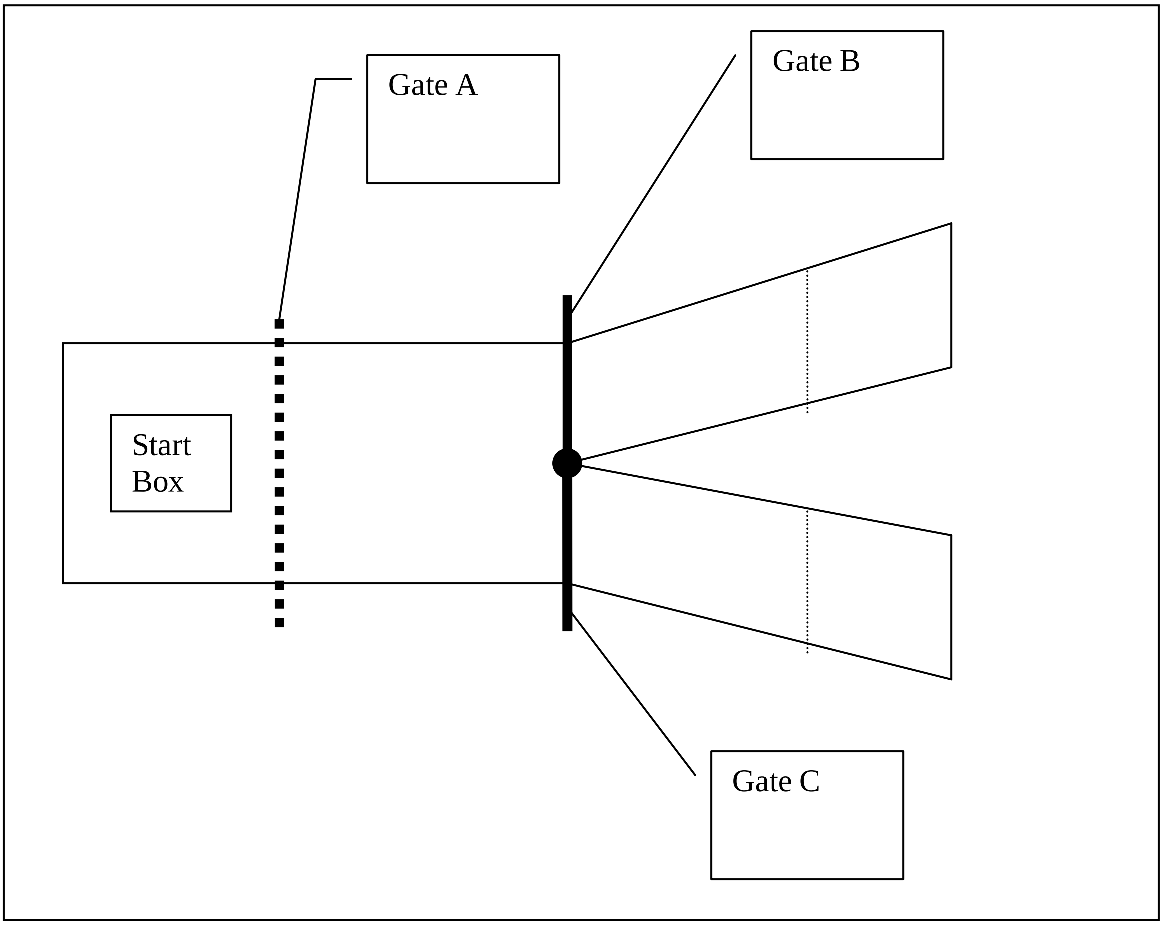


**Figure 1: Diagram of the Y-maze test apparatus**

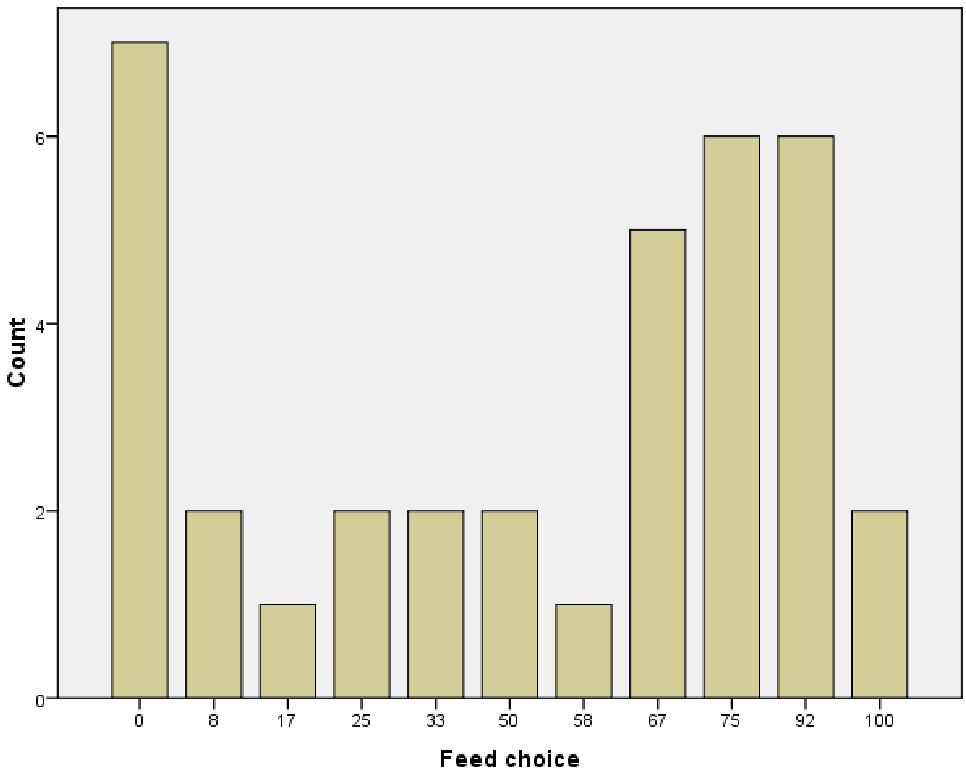


**Figure 2: Histogram of feed choice (% trials in which feed was chosen) of the 36 pigs in Experiment 1.**

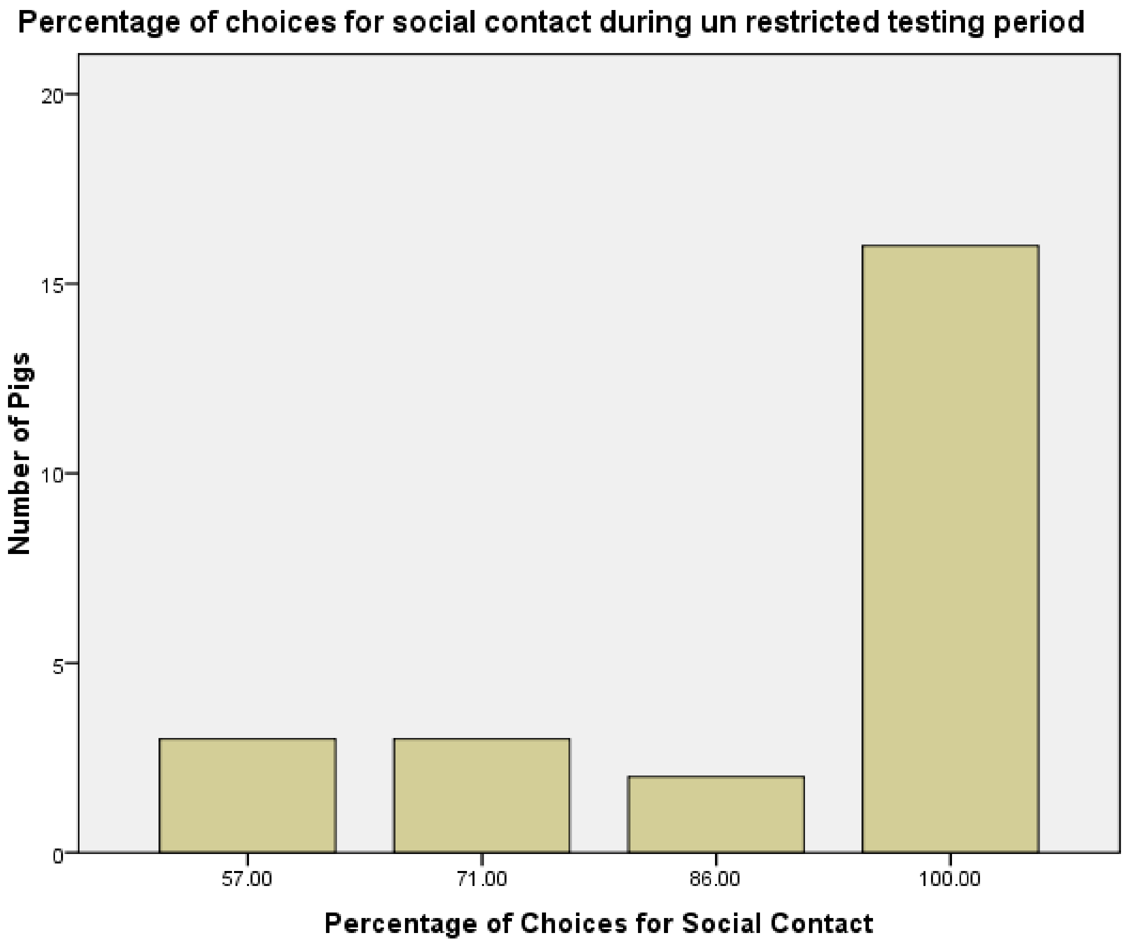


**Figure 3: Histogram of Social contact choice (% trials in which feed was chosen) of the 36 pigs Experiment 2 during initial 7 trials when animals were not restricted in their home pens.**

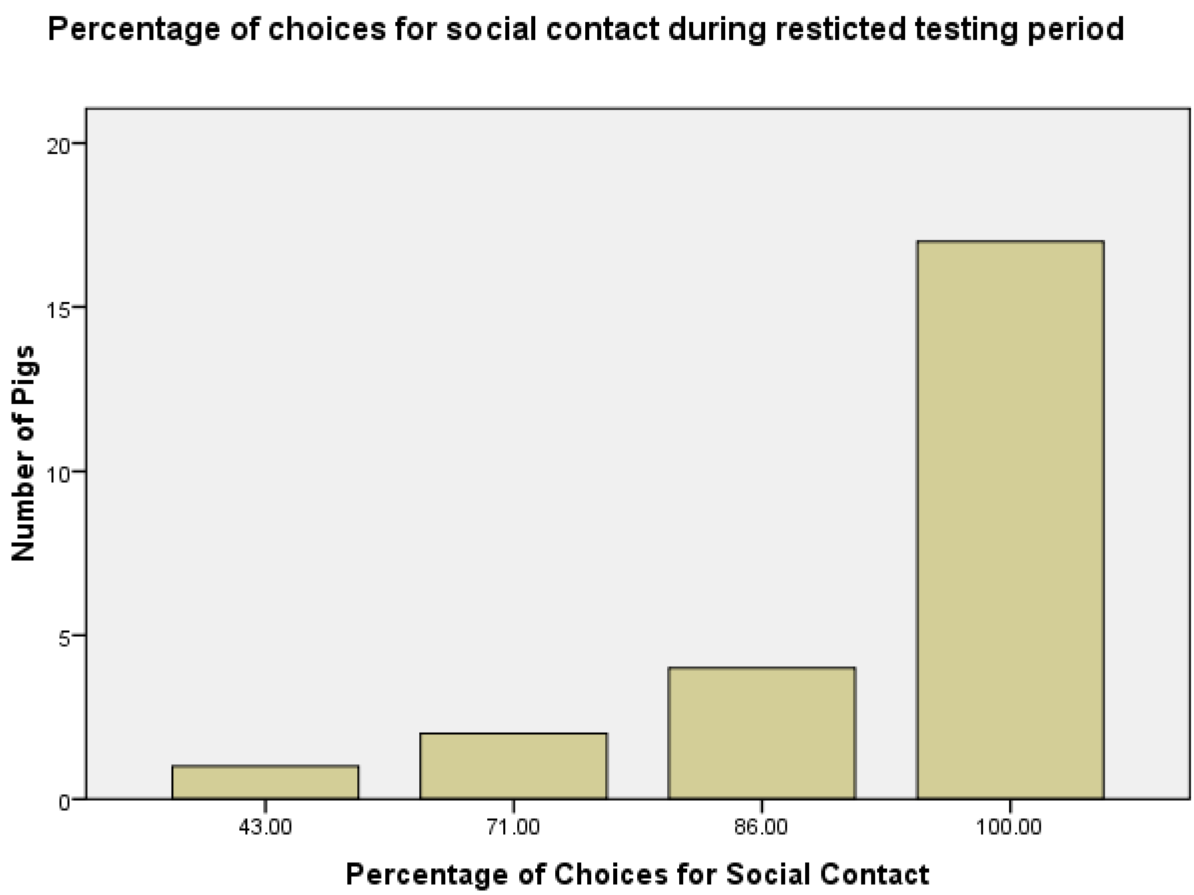


**Figure 4: Histogram of Social contact choice (% trials in which feed was chosen) of the 36 pigs Experiment 2 during the 7 trials when animals were restricted of social contact and environmental enrichment in their home pens.**

**Table 1: Effects of restriction (feed or social) and preference (feed or social) on pig behaviour and live weight in Part 2, Experiment 1. Estimated marginal means of the frequency of occurrence of postures and behaviours are presented (behaviour variables presented as frequency of the behaviour as observed with instantaneous sampling at 15-min intervals over 25 h).**

| Variable | Restriction | | P value | Preference | | P value |
|---|---|---|---|---|---|---|
| | Feed | Social | | Feed | Social | |
| Lying[1] | 1.90 | 1.89 | 0.691 | 1.88 | 1.91 | 0.314 |
| Standing[1] | 1.35 | 1.28 | 0.502 | 1.38 | 1.28 | 0.141 |
| Sitting | 3.38 | 5.75 | 0.307 | 3.50 | 5.62 | 0.359 |
| Interact with feeder | 11.13 | 13.78 | 0.067 | 11.75 | 13.13 | 0.313 |
| Interact with drinker | 2.13 | 1.88 | 0.785 | 2.13 | 1.88 | 0.785 |
| Interact with floor | 5.75 | 7.37 | 0.594 | 8.50 | 4.63 | 0.216 |
| Interact with pig[1] | 0.29 | 0.00 | 0.000 | 0.08 | 0.21 | 0.035 |
| Interact with pen fittings | 4.25 | 4.63 | 0.847 | 4.63 | 4.25 | 0.874 |
| Final weight (kg)[2] | 162.3 | 165.2 | 0.681 | 165.7 | 161.8 | 0.579 |

[1]Data log(Y+1) transformed prior to analysis and the back transformed means presented.
[2] Initial live weight in Part 2 used as covariate.

**Table 2: Interactions between main effects on final live weight (P=0.032) and free cortisol concentration (P=0.110) in Part 2, Experiment 1.**

| Variable | Treatment | Preference | Weight (kg) |
|---|---|---|---|
| Final live weight (kg) | Feed restriction | Feed preferred pigs | 155.9 |
| | | Social preferred pigs | 168.8 |
| | Social restriction | Feed preferred pigs | 175.5 |
| | | Social preferred pigs | 154.9 |
| Free cortisol concentration (ng/ml)[1] | Feed restriction | Feed preferred pigs | 0.65 |
| | | Social preferred pigs | 0.36 |
| | Social restriction | Feed preferred pigs | 0.31 |
| | | Social preferred pigs | 0.34 |

[1] Day-time average concentration and data log(Y+1) transformed prior to analysis and the backtransformed means presented.

**Table 3: Effects of preference (feed or social) and restriction (feed or social) on the stress physiology of pigs in Experiment 1. Cortisol concentrations and estimated marginal means presented.**

| Variable | Restriction | | P value | Preference | | P value |
|---|---|---|---|---|---|---|
| | Feed | Social | | Feed | Social | |
| Total cortisol concentration (ng/ml)[1] | 22.6 | 14.0 | 0.044 | 16.6 | 20.1 | 0.381 |
| Free cortisol concentration (ng/ml)[2] | 0.50 | 0.33 | 0.079 | 0.48 | 0.35 | 0.209 |
| Peak cortisol response to ACTH (ng/ml)[3] | 135.1 | 121.0 | 0.210 | 138.5 | 117.5 | 0.072 |
| Peak cortisol response to CRH (ng/ml)[3] | 53.2 | 27.3 | 0.013 | 38.1 | 42.4 | 0.625 |

[1] Day-time average concentration

[2] Day-time average concentration and data log(Y+1) transformed prior to analysis and the backtransformed means presented.

[3] Peak total cortisol concentration within 9 h after the ACTH or CRH injection

**Table 4: Effects of restriction (environmental enrichment or social contact) and preference during restricted testing period (environmental enrichment or social contact) on pig behaviour and live weight in Part 2, Experiment 2. Estimated marginal means of the frequency of occurrence of postures and behaviours are presented (behaviour variables presented as frequency of the behaviour as observed with instantaneous sampling at 15-min intervals over 14 h).**

| **Variable** | **Restriction** | | **P** | **Preference** | | **P** |
|---|---|---|---|---|---|---|
| | **Enrichment** | **Social** | **value** | **Enrichment** | **Social** | **value** |
| Lying | 0.682 | 0.668 | 0.668 | 0.664 | 0.686 | 0.496 |
| Standing* | 0.289 | 0.265 | 0.454 | 0.283 | 0.271 | 0.704 |
| Sitting | 0.030 | 0.066 | 0.148 | 0.053 | 0.043 | 0.689 |
| Interact with feeder | 0.116 | 0.132 | 0.414 | 0.105 | 0.142 | 0.071 |
| Interact with drinker | 0.029 | 0.030 | 0.911 | 0.029 | 0.030 | 0.935 |
| Interact with floor[1] | 0.094 | 0.109 | 0.458 | 0.104 | 0.099 | 0.806 |
| Interact with pig[1] | 0.019 | 0.000 | **0.007** | 0.012 | 0.007 | 0.498 |
| Interact with pen fittings | 0.034 | 0.030 | 0.733 | 0.038 | 0.026 | 0.355 |
| Interaction with environmental enrichment[1] | 0.000 | 0.009 | **0.006** | 0.007 | 0.005 | 0.448 |
| Final weight (kg)[2] | 101.3 | 99.3 | 0.733 | 97.9 | 102.8 | 0.448 |

[1]Data log(Y+1) transformed prior to analysis and the backtransformed means presented.

[2] Initial live weight in Part 2 used as covariate.

*Interaction between main effects, see table 5.

**Table 5: Interactions between main effects using preferences from the restricted testing period on final live weight (P=0.051), free cortisol concentration (P=0.225), peak cortisol response to CRH (0.846) and peak ACTH response to CRH (0.172) in Part 2, Experiment 2.**

| Treatment | Preference | Final Live Weight (kg)[1] | Total Cortisol (ng/ml)[3] | Free Cortisol (ng/ml) | Peak Cortisol to CRH(ng/ml)[4] | Peak ACTH to CRH (ng/ml)[4] | Standing (proportion of observations) |
|---|---|---|---|---|---|---|---|
| Enrichment restriction | Enrichment preferred pigs | 105.1 | 17.05 | 1.105 | 67.61 | 45.60 | 0.326 |
| | Social preferred pigs | 97.6 | 13.06 | 0.973 | 62.09 | 58.88 | 0.251 |
| Social restriction | Enrichment preferred pigs | 90.6 | 14.70 | 1.203 | 74.30 | 47.32 | 0.240 |
| | Social preferred pigs | 107.9 | 10.22 | 0.969 | 50.46 | 60.40 | 0.291 |
| P Value | | **0.047** | 0.863 | 0.836 | 0.488 | 0.799 | **0.051** |

[1] Initial live weight in Part 2 used as covariate.

[2] Total feed calculated from the total food given minus refusals for the period from entry to part 2 to removal post CRH challenge.

[3] Day-time average concentration and data log(Y+1) transformed prior to analysis and the backtransformed means presented.

[4] Peak total cortisol or ACTH concentration within 4h after the CRH injection and data log(Y+1) transformed prior to analysis and the backtransformed means presented

**Table 6: Effects of restriction (environmental enrichment or social) and preference during the restricted testing period (environmental enrichment or social) on the stress physiology of pigs in Experiment 2. Cortisol concentrations and estimated marginal means presented.**

| Variable | Restriction | | P | Preference | | P |
|---|---|---|---|---|---|---|
| | Enrichment | Social | Value | Enrichment | Social | value |
| Total cortisol concentration (ng/ml)[1] | 14.922 | 12.274 | 0.466 | 15.827 | 11.560 | 0.250 |
| Free cortisol concentration (ng/ml)[2] | 1.040 | 1.086 | 0.852 | 1.154 | 0.972 | 0.464 |
| Peak cortisol response to ACTH (ng/ml)[3] | 240.9 | 241.7 | 0.412 | 246.8 | 208.8 | 0.240 |
| Peak cortisol response to CRH (ng/ml)[4] | 64.86 | 61.24 | 0.794 | 70.96 | 55.98 | 0.284 |
| Peak ACTH response to CRH (pg/ml)[5] | 48.19 | 53.46 | 0.690 | 46.45 | 55.46 | 0.497 |

[1] Day-time average concentration and data log(Y+1) transformed prior to analysis and the backtransformed means presented

[2] Day-time average concentration.

[3] Peak total cortisol concentration within 4h after the ACTH injection

[4] Peak total cortisol concentration within 4h after the CRH injection and data log(Y+1) transformed prior to analysis and the backtransformed means presented

[5] Peak ACTH concentration within 4h after the CRH injection and data log(Y+1) transformed prior to analysis and the backtransformed means presented