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AUSTRALIAN Pork

Enrichment in the sucker and weaner phase altered exploratory behaviour and the immune system of pigs.

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Executive Summary

This project aimed to evaluate the provision of enrichment to pigs in the sucker and weaner phases. Pigs were provided with enrichment blocks (Ridleys Corporation) that were specifically formulated for use in young pigs or were not provided with any added enrichment. A 2 x 2 factorial design was repeated over four replicates in time. We tested the unifying hypothesis that provision of enrichment in the form of enrichment blocks during the sucker and weaner phases would have benefits for the welfare of the pigs. The benefit of the provision of enrichment on the behaviour of the pigs was evaluated by measuring the performance of the pigs in an open field/novel object test, a maze test and an executive function test. The benefit of the provision of enrichment on the immune system of the pigs during the first ten-weeks of life and the effect of enrichment on the cortisol response of the pigs after exposure to an open field test was also assessed.

The provision of enrichment blocks altered the behaviour of the pigs in the novel object/open field test, the maze test and the executive function test and these changes suggest an increased willingness to explore and possibly an increased ability to learn. The behavioural tests highlighted that young pigs have the capacity to learn complex tasks. The provision of enrichment attenuated the production of tumour necrosis factor α (TNFα) in response to weaning and this indicated an attenuated pro-inflammatory response to weaning. In addition, there was an overall effect of the provision of enrichment on haemoglobin, haematocrit, red blood cell distribution width and the number of platelets. This is further evidence of an attenuated inflammatory response in pigs provided with enrichment. There was no difference in the cortisol response to a novel arena based on the provision of enrichment.

The current project has identified that enrichment in the sucker and weaner phase can affect the behaviour of pigs, can affect their ability to learn and affect their immune system and response to weaning. These data support the notion that the benefits of enrichment cannot be accurately gauged by measuring the interactions the animal has with the enrichments in the home pen and it may simply be beneficial to live in a more complex environment. Although this project has not identified one clear benefit of the provision of the enrichment blocks it has identified that enrichment provided in the sucker phase did have benefits for the piglet and that enrichment provided in the weaner phase also had benefits. The overall implication of this research is that environmental enrichment likely impacts the behaviour, learning ability and immune function of young pigs. The current project could not identify what the longer term implications of these changes were for the welfare of the pigs, however, we speculate that the pigs provided with enrichment would be better prepared to cope with challenges and may adapt faster to new environments. We have highlighted that the early rearing environment is important and that the management and husbandry at an early age can have long term implications for pigs. The enrichment we used in this study was very simple, an enrichment block, and we have evidence that suggests the provision of enrichment effected the behaviour and the immune system of the pigs. Even the simplest of enrichments may have benefits for the welfare and development of young pigs and there is merit in developing enrichment devices that are suitable for use in the Australian pig industry.

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1. Background to Research

This research project was developed to address the issue of welfare interventions for pigs, and specifically to investigate whether the application of some practical enrichment stimulations for pigs during the sucker and weaner stages of production provide measurable welfare benefits to the pig. The research aimed to investigate whether stimulation applied in the sucker and weaner stages has benefits for the welfare of pigs as measured by learning ability, latency to explore, function of the immune system and cortisol response to a novel arena.

Enrichment of the environment is seen as an approach which may benefit the welfare of pigs of all ages. Public expectation is that pigs raised commercially are housed in such a way that they are able to perform their species-specific behavioural repertoire. This is problematic in Australia, since the typical indoor, pig-pen environment is constructed of durable materials such as concrete and metal, does not contain bedding material, such as straw, and hence lacks enrichment. Thus, pig pens often lack enrichment and are considered 'barren'. Pigs housed in barren pens can be perceived as having compromised welfare compared to pigs housed in enriched pens. There is, however, little evidence to support either perception. The current research proposal followed on, in part, from research at the University of Sydney, in which the vitality, neonatal behavioural and physiological characteristics of piglets born in farrowing crates or farrowing pens was assessed in relation to lifetime growth performance. This project built on the previous and current research, and addressed Australian Pork Limited's Core Objectives of growing consumer appeal and leading sustainability by identifying Australian pork as being produced from pigs that are housed and husbanded in an ethical manner, and thus being more popular with consumers.

Environmental enrichment for pigs may be considered from two perspectives: (perspective 1) the complexity of the available space, and (perspective 2) the opportunity of the pigs to behave in an appropriate, "species-specific" manner.

Perspective 1 - specifically focusses on the lactation environment, in which suckers may experience a range in spatial complexity, from less complex, for example involving the sow being housed in a crate and thus less able to interact with her piglets, to higher complexity, for example involving the sow being "loose" and thus enabling the sow to interact more with her piglets.

Perspective 2 - relates more to behaviours that are species-specific. For the pig this often relates to the performance of oro-nasal activities such as root-nose-chew-ingest. These are components of porcine foraging behaviour. Researchers therefore have suggested that pigs find objects that are 'ingestible', 'odorous', 'chewable', 'deformable' and 'destructible' to be enriching. Although commercial pigs are group-housed during most stages of production, which provides the opportunity for enrichment in the form of (some) spatial complexity, enrichment is often limited to social stimulation from other pigs. The barren pen environment and associated lack of stimulating objects are seen as deficiencies which could compromise pig welfare. Thus, there is a strong push to provide pigs with enrichment, not just in the form of space and, or social contact, but in the form of manipulable objects or bedding that stimulates the pig and potentially improves the welfare of the pig

While the literature does not provide conclusive evidence that the provision of enrichment improves pig welfare outcomes, there is nevertheless a strong belief that if pigs can perform more of their species-specific repertoire, this equates to improved welfare. Environmental enrichment research in pigs has typically focussed on increasing species-specific behaviours associated with foraging activity.

That is, researchers have aimed to increase the frequency of particular behaviours by pigs, as well as increase the diversity of behaviours performed. However, this approach may only be successful under certain circumstances. For example, if we assume that for commercially-housed pigs to have 'good' welfare, their ethogram (i.e. the full range of possible behaviours performed) should resemble that of a similar pig in a 'naturalistic' environment, we must first be cognisant that wild animals vary their behaviour in response to the local environmental conditions. Increased behavioural complexity therefore, is not necessarily a suitable measure of welfare. The range of a pig's behavioural responses in a controlled indoor environment will necessarily be limited by the lack of diversity of stimuli. Extreme stimuli, such as would occur upon the appearance of a predator, is avoided by housing pigs appropriately (e.g. in an environment safe from predators, parasites and exposure to diseases, with regular nutritious feed, *ad libitum* water supply, and in which the pigs are able to avoid extremes of climate variation). Thus, the relevance of the stimulus to the animal needs to be considered when providing environmental enrichment. Further, animals usually habituate to repeated presentation of a stimulus that is not relevant for survival. Hence, pigs often ignore enrichment devices which have been provided to them for extended periods.

Food is an important motivational factor for pigs. Indeed, food is often a more important motivator than social contact for pigs. Foraging, which is a syndrome or series of appetitive (goal-seeking) behaviours whereby the pig investigates its environment, is performed for the purpose of finding food to ingest (consummatory behaviour). Hence, when considering enrichment of the pigs' environment, it is appropriate to stimulate this appetitive phase of foraging behaviour in order to maintain higher levels of motivation so that the pigs continue to utilise the enrichment provided, and to avoid habituation to the stimulus. In this project we proposed to incorporate a sensory stimulus to motivate foraging behaviour.

Thus, this project was developed to investigate different, practical forms of environmental enrichment for pigs up to 10 weeks of age and determine whether the enrichment provides benefits for pig welfare and production. The research applied enrichment to young (sucker) pigs in the lactation environment and then applied enrichment to pigs in the weaner environment. Enrichment was in the form of enrichment blocks. Welfare variables that were measured focussed on pig behavioural responses in an open field test, a maze test and an executive function test in addition to the immune capability and cortisol response. We tested the unifying hypothesis that provision of enrichment in the form of enrichment blocks in sucker and weaner phase would have benefits for the welfare of pigs.

2. Objectives of the Research Project

Determine whether added enrichment provided for pigs between 1 and 10 weeks of age provides welfare, health and production benefits measured in the sucker and weaner phases of production.

3. Introductory Technical Information

Environmental enrichment is the modification of a barren captive-environment to improve the biological functioning of animals [1]. Enrichments can enhance the well-being of animals by allowing them to perform more of their species-specific behavioural repertoire and accommodate a larger range of behavioural choices [2]. Enrichments generally provide novelty, social contact and exercise that is rewarding and results in net overall benefit for the animal [1]. Whilst environmental enrichment is accepted as improving the wellbeing of animals, animals housed in enriched environments have greater glucocorticoid concentrations than animals housed in barren environments [3]. Therefore, assessing the welfare benefits of environmental enrichment poses a number of challenges.

Evaluating the benefit of environmental enrichment is challenging, however, animals housed in enriched environments can be less anxious, engage in more social contact, cope better with stress and have stronger immune systems. Meehan and Mench [4] referred to environmental enrichment as a positive stressor and acknowledged, that the lack of challenge in an environment is as detrimental to welfare as too much challenge. Moreover, when challenge is applied in a species specific and appropriate manner the welfare of the animal is improved. In 1976 Selye introduced the concept of eustress and distress with distress being stress that has a negative effect and eustress being stress that has a positive effect [5]. Selye described how eustress would enable adaptation and make an animal better able to cope whilst distress would lead to a pathological state. Environmental enrichment could be viewed as eustress in that it enables adaptation and by doing so equips animals to cope better with subsequent stressors. Croften et al [1] describes environmental enrichment as inoculation stress. It is a process by which animals develop resilience to future stressful experiences by first being exposed to mildly stressful events early in life [1, 6]. Evidence in range of species supports this and indicates that the best way to evaluate the benefit of environmental enrichment is to test how animals in enriched environments cope with experiences outside of their home pen.

Laboratory mice raised in enriched cages had significantly greater basal corticosterone concentrations than mice raised in barren cages [3]. When exposed to a stress paradigm, elevated plus maze and staircase, mice from enriched cages showed no significant increase in plasma corticosterone whereas mice from barren cages did show a significant increase in corticosterone [3]. Mice from enriched cages explored the maze more, were more active and had greater natural killer cell activity than controls [3]. In a similar study it was shown that neonatal handling enabled adult mice to cope better with a swim test [7]. Pigs that are kept on deep litter had greater 24 h salivary cortisol than pigs that were kept in barren cages and this has been shown in a number of experiments [8, 9]. In addition, pigs exposed to an enriched rewarding environment containing straw and chocolate raisins had a significant increase in cortisol that was very similar to the cortisol response of pigs exposed to an aversive environment consisting of a barren concrete floor and intermittent snout roping [10]. Pigs housed in barren conditions had greater cortisol responses to transport, handling and lairage than pigs housed on straw [8, 9, 11]. Pigs housed in barren environments experienced greater cortisol responses during slaughter than pigs housed in enriched environments [9].

Environmental enrichment enhances the welfare of animals, activates the hypothalamo-pituitary adrenal (HPA axis) and the sympathoadrenal system whilst the brain of animals housed in enriched environments undergoes molecular and morphological changes that lead to enhanced learning, memory and ability to cope with stress [12]. Assessing the value of environmental enrichment is challenging and we propose that the main benefit of enrichment to the welfare of pigs is an enhanced ability to learn, enhanced ability to cope with stressors and enhanced immune competence. This has

multiple benefits for the pig and for the pig industry because if appropriate enrichment is provided animals may be more resilient, be less susceptible to disease, be easier to move and to handle as well as having benefits for their welfare. There is little research published on the benefits of enrichment to pigs in this context.

4. Research Methodology

A 2 X 2 factorial experiment, replicated over 4 time periods, was conducted at Roseworthy Piggery, Roseworthy SA, to investigate the effects of enrichment in the sucker phase and in the weaner phase with measurement of behavioural, health and immune responses relevant to welfare and production, as well as to investigate the pigs' utilization of enrichment during the pre- and post-weaning period. All procedures were approved by the Department of Primary Industries and Regions South Australia Animal Ethics Committee (AEC project number 34/15).

Piglets (Large White × Landrace) were housed in conventional farrowing crates for 21 days during lactation and then in group (weaner) pens until 11 weeks of age. Litters (n=96) were randomly assigned to treatments within a 2 × 2 factorial design, with four replicates in time. Four focal pigs were selected from each litter (2 males and 2 females) for experimental observation. The pigs were raised with either enrichment (E) or no enrichment (barren: B) provided for the crate/pen, with cross-over between the sucker and weaner phases. At weaning, the four focal pigs per litter were grouped in weaner pens with a total of 24 weaners per replicate of the same treatment grouping (See Figure 1.). Thus there were four treatments: enriched in sucker phase and enriched in weaner phase (EE; n=96), enriched in the sucker phase and barren in the weaner phase (EB; n=96), barren in the sucker phase and enriched the weaner phase (BE; n=96) and barren in the sucker phase and barren in the weaner phase (BB; n=96). Food and water were provided *ad libitum* during the weaner phase. Enrichment blocks were replaced weekly and increased in size to match piglet size.

A series of tests were conducted throughout the 11 weeks and these are described in detail below. Pigs were weighed weekly throughout the 11 weeks and were scratch and injury scored weekly and one day after weaning. At day 31 of life 6 pigs per replicate (24 pigs total) were exposed to an open field/novel object test, at day 56 of life 6 pigs per replicate (24 pigs total) were exposed to a maze test, at day 73 of life 6 pigs per replicate (24 pigs per replicate) were exposed to an executive function test and at day 78 of life 6 pigs per replicate were exposed to an open field/novel object test where blood samples were collected every 15 min for 2 h prior to the test and every 15 min for 2 h after the test. The experiment was designed such that each pig was only exposed to one test, no pigs were exposed to multiple tests. During replicate 4 a series of blood samples were collected to evaluate the immune function of the animals.

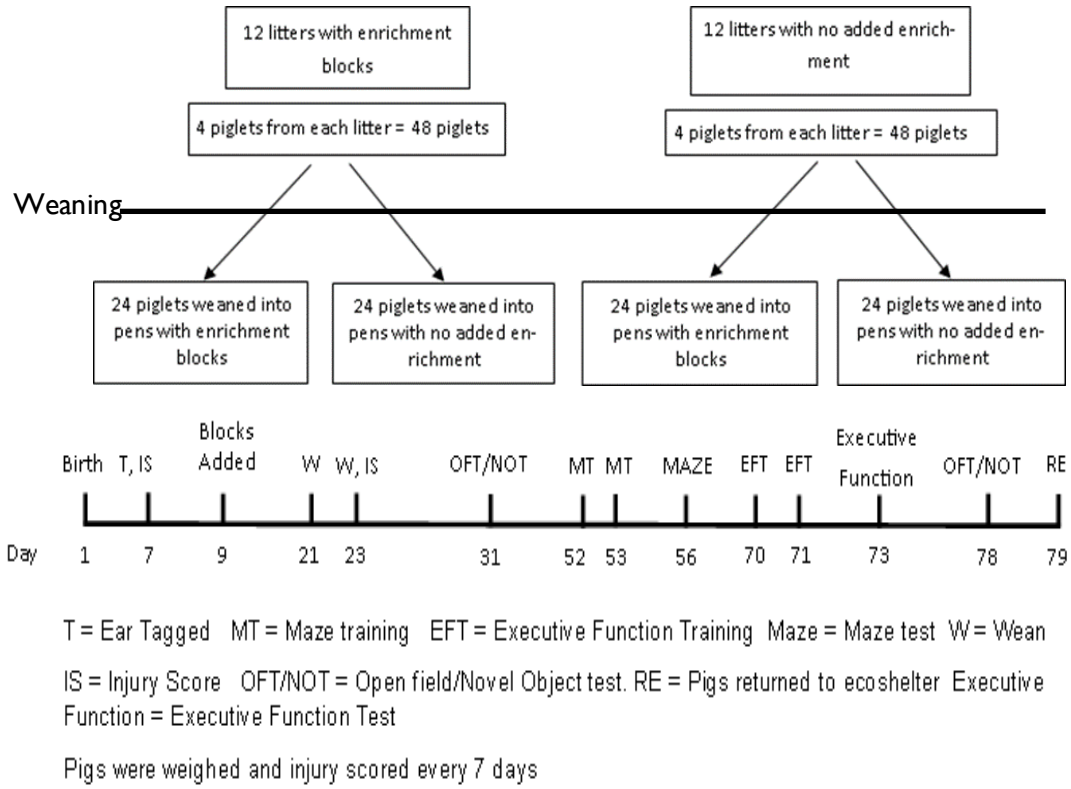

Figure 1. The experimental design for the experiment. The top panel depicts the provision of enrichment in the sucker and weaner phases and the 2 x 2 factorial design. The bottom panel shows that time line of events in each replicate of the experiment.

4.1 Open Field/Novel Object Test

On day 31 of life 6 pigs per replicate (24 pigs total) were exposed to an open field test/novel object test. Figure 2 depicts the testing aparatus. The piglet was placed inside the start box and after 1 min was provided opportunity to emerge from the start box into the arena. Emergence time from the start box was recorded and then behaviours listed in Table 1 were recorded in real time. After 3 min in the arena a novel object (red bucket) was introduced and the piglet remained in the arena for a further 2 min. At the conclusion of the test the piglet was returned to it's home pen.

Table 1. Ethogram of behaviours recorded in the open field and novel object test

Behaviour	Description
Emergence	Head and front shoulders cross starting box threshold.
Zone Crossed	Head and front shoulders cross over marked lines into a new zone.
Grunts	One low frequency sound produced by the pig or for succussive grunting, counted for every 5 seconds it continued
Squeal	A high frequency noise produced by the pig
Urination	Pig expels urine inside the testing arena
Defecation	Pig expels faeces inside the testing arena
Jump at wall	Launches body at walls of testing arena
Interaction	Sniff/ softly touch novel object with snout
Knock	Forceful hit with the swing of the head
Avoid	Actively avoids novel object when moving around the arena

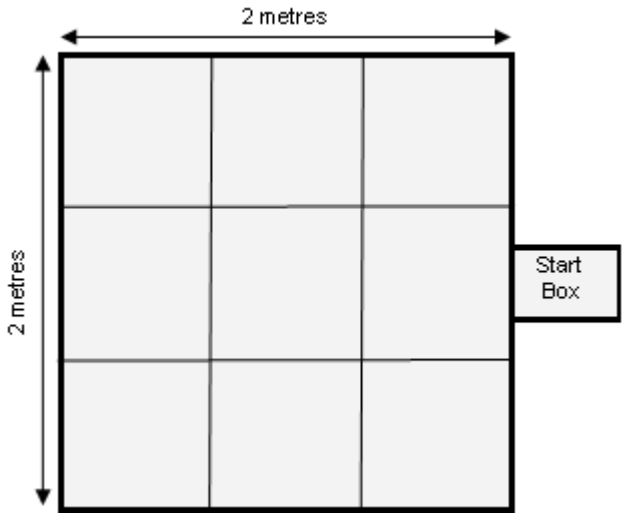

Figure 2. Test arena for Open Field and Novel Object Test

4.2 Maze Test

At 56 days of life 6 pigs per replicate, 24 pigs in total were exposed to a maze test. The maze testing apparatus is shown in Figure 3. The test consisted of two phases. The training phase (conducted on day 52 and day 53 of life) and the testing phase (conducted on day 56 of life).

4.2.1 Training Phase

The test arena consisted of 2 traps with a start box at one end of the maze and 2 familiar pigs and the reward (canned cream in a bowl) at the other end of the maze. The start box in the maze test had a transparent door such that the pigs could see the maze while in the start box. Pigs were exposed to 3 training runs to learn the maze and then two days later were exposed to 4 test runs. Each pig was held in the start box for 1 minute and then released into the maze. Researchers recorded time to exit the start box, time taken to navigate through the maze and reach the reward, the number of times

that pig entered a trap and the total time spent in the trap (Table 2). The difference in time taken to solve the maze between test 1 and test 4 was also evaluated.

Table 2. Ethogram of behaviours recorded in the maze test.

Behaviour	Description
Emergence	Head and front shoulders cross starting box threshold.
Reach Reward	Snout touches bowl that contains the reward or engages in eating the reward.
Trap	Head and front shoulders cross over the line marking the entrance of the trap, considered 'trapped' until head and shoulders cross back over the entrance line.
Urinate	Piglet expels urine inside the testing arena.
Defecate	Piglet expels faeces inside the testing arena

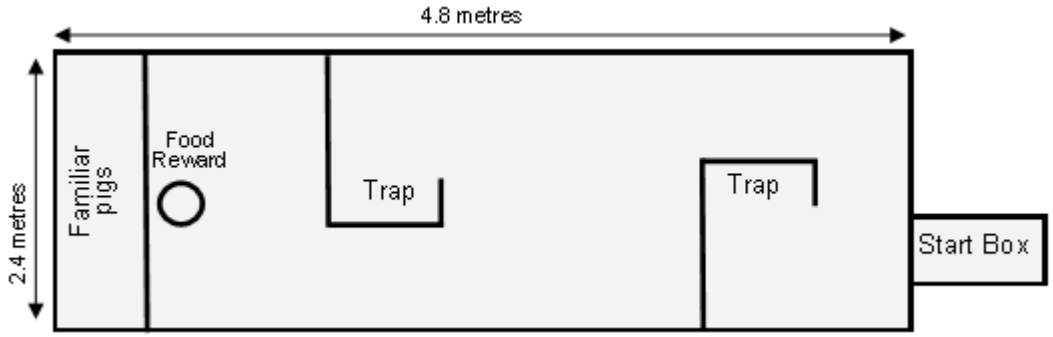

Figure 3. Test arena for the Maze Test.

4.3 Executive Function Test

The executive function test assessed the ability of the animal to learn an audible cue and associate that cue with a food reward. The arena used in the executive function test is shown in Figure 4. The test consisted of two phases the training phase and the testing phase.

Training phase: (1) habituation of the group of focal pigs to the arena
The pigs were introduced to the arena in a group of six through the start box. They remained in the test arena for 5 minutes and were allowed to freely explore the arena. The group of 6 pigs was then re-introduced to the test arena through the start box and again were allowed 5 min to freely explore the test arena. They were then quietly moved out of the test arena via exit gate B. There were four groups of pigs trained each day. Group one exited from gate A first, group two exited from gate B first, group three exited from gate A first and group four exited from gate B first. This ensured we were not introducing a bias to the pigs by moving all of them through exit gate A first.

Training phase: (2) habituation of individual focal pigs to the procedure
The aim of this phase was to determine the natural inclination of the pig to turn left or right and condition the pig to associate a sound with a food reward.

A food reward was placed at both ends of the arena. The pig was held in the start box for 10 seconds (s) and the sounds became audible to the pig after 8 s in the start box i.e. 2 s prior to being released from the start box. For 3 focal pigs sound A was broadcast from speaker 1 and sound B was broadcast from speaker 2 (Figure 4). Both sounds were broadcast at the same time. For the other 3 focal pigs in the group, the positions of the sounds were reversed.

Time taken to exit the start box, movement in the arena until the pig crossed either the end zone line (i.e. enters zone 1 or 5 (Figure 4)) or made contact with either the bowl containing the food reward or the bowl that did not contain the food reward was recorded. Also recorded was which end of the arena the pig first moved towards, and where it first reached the end line / food reward. Once the pig reached one end of the test arena it was allowed to exit the arena via the exit gate at the end it reached. Each pig was exposed to this procedure 3 times on training day 1 and 4 times on training day 2.

This training phase enabled the researcher to determine which side of the arena the pig was naturally inclined to turn towards (if the pigs preferred the left side or the right side) and train the pig to associate the food reward with the sound located at the end of the arena that the pig preferred (the pigs preferred sound). This information was recorded and is critical to the testing phase.

4.3.1 Testing Procedure – Executive Function Test

Each focal pig was tested individually three times across the testing day. The pig was held in the start box for 10 s and the sounds became audible to the pig after 8 s in the start box i.e. 2 s prior to being released from the start box.

During the test the pigs were presented with both sounds and the sound that indicates food reward was broadcast from the opposite side to their previously determined preference. The preferred sound for the respective pig was presented on the pig's non-preferred side and the food reward was only available at the side of the arena where the preferred sound was located. For example, if it was determined during the training phase that the pig preferred side A and sound 1 then during the testing phase sound 1 and the food reward would be placed at side B. The time taken to exit the start box, movement in the arena until the pig crosses either the end zone line (i.e. enters zone 1 or 5 in Figure 4) or makes contact with either the bowl containing the food reward or the bowl without the food reward was recorded (Table 3). If none of these occurred the test was terminated after 2 minutes. At the end of each test the test pig was quietly removed from the end where the cream was located.

Table 3. Ethogram of behaviours recorded in the executive function test.

Behaviour	Description
Emergence	Head and front shoulders cross starting box threshold
Zone Entered	Head and front shoulders cross over marked lines into the adjacent zone
Bowl Reached	Snout touches bowl or cream reward in bowl

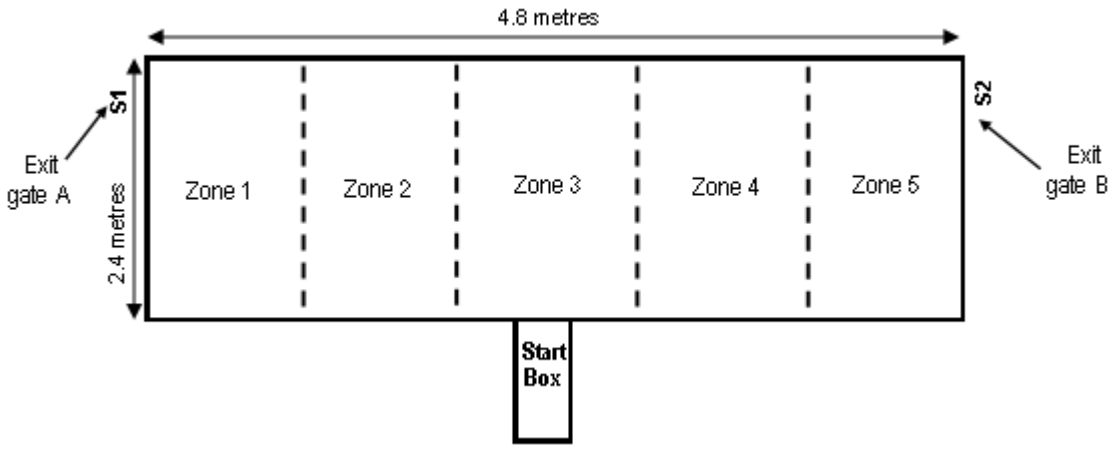

Figure 4. Test arena for Executive Function Test. S1 depicts speaker 1 and S2 depicts speaker 2.

4.4 Cortisol Response to Novel Object/Open Field Test

This test was applied as described in section 6.1 above. Indwelling ear vein catheters were implanted in the pigs at 10:00 h on the morning of the test and the pigs were given 4 hours to recover from the procedure. Blood samples were collected 120 min, 90 min, 60 min, 30 min, 15 min and 1 min prior to the test and then 15 min, 30 min, 60 min, 90 min and 120 min after the completion of the open field test. Blood samples were centrifuged at 3000 rpm for 10 min, plasma harvested and frozen at -20°C until analysis. Plasma was assayed for cortisol using radio-immuno assay (MP Biomedicals).

4.5 Immune Function

The effect of enrichment on the immune competence of the pigs was assessed in replicate four only.

4.6 Blood collection and processing

All products were purchased from R&D systems (Minneapolis, MN, United States) unless otherwise stated. Six ml blood samples were collected via jugular venepuncture into EDTA anticoagulant vacutainer tubes (Becton Dickenson; North Ryde, NSW, Australia) at 1 day prior to weaning, 1 day post weaning, 21 days post weaning, and 56 days post weaning. Whole blood was maintained at 23°C for a maximum of 5h until immune cell separation or complete blood count, then maintained on ice until plasma collection. Samples were centrifuged at 1500*g* for 15 min at 4°C and plasma harvested. Plasma was collected and stored at -80°C in duplicate for enzyme-linked immunosorbent assay (ELISA) analysis. Peripheral mononuclear blood cells (PMBCs) were isolated from whole blood using density gradient centrifugation. Blood samples (3 ml) were gently layered over a density gradient medium (4.5ml, Lymphoprep; STEMCELL Technologies Australia Pty. Ltd.; Tullamarine, VIC Australia) in specialised conical tubes (Sep Mate 15; STEMCELL Technologies) and centrifuged at 1200 *g* for 10 min. PMBCs were washed twice in phosphate buffered saline (PBS; Sigma-Aldrich Pty. Ltd. Sydney, NSW, Australia) containing 0.5% heat inactivated Gibco foetal calf serum (FCS; *Thermo Fisher Scientific Australia Pty Ltd;* Scoresby, VIC Australia) and centrifuged at 300 *g* for 10 min. PMBCs were resuspended in 1ml RPMI-1640 (Sigma Aldrich) containing 10% FCS (herein defined as cell medium) for cell concentration assessment on a Cell-Dyn 3700 hematology analyser (Abbott Diagnostics; North Ryde, NSW Australia). Cell preparations were diluted to 1×10^6 cells/ml with cell medium and stored at 37°C 5% CO_2 until enzyme-linked immunospot (ELISPOT) assessment.

4.7 Complete blood count

Aliquots (0.5ml) of whole blood were taken for a complete blood count on a Cell-Dyn 3700 hematology analyser. Samples were processed as per manufacturer's instructions. Haematologic variables examined were total white blood cell, lymphocyte, monocyte, and neutrophil counts as well as red blood cell count (RBC), haemoglobin (HGB), haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), red blood cell distribution width (RDW), mean corpuscular haemoglobin concentration (MCHC), and platelet number (PL).

4.8 Enzyme-linked Immunosorbent Assay (ELISA)

Cytokine concentrations for IL-1β, interleukin – 2 (IL-2), interleukin – 6 (IL – 6), interleukin – 10 (IL-10), interleukin – 12 (IL-12), tumor necrosis factor alpha (TNF-α), and IFNγ were measured in plasma using commercially available porcine DuoSet® ELISA kits as per manufacturer's instructions. Standards were diluted in PBS containing 10% FCS instead of 1% BSA for matrix continuity between standard curves and samples as per manufacturer's recommendation. Samples were assessed in triplicate, intraassay variation was <10%, and interassay variation was < 15%. Briefly, 96 well plates were incubated with capture antibody overnight at 23°C. Plates were blocked with 1% BSA in PBS for 1h. Standards, controls, and samples (100ul) were incubated for 2h at 23°C. Plates were incubated with biotinylated detection antibody for 2h at 23°C protected from light. Plates were incubated with Streptavidin HRP (1:200) for 20min at 23°C protected from light. Plates were incubated with TMB substrate solution (Mabtech) for 30 min at 23°C protected from light. Reactions were stopped with 1N H_2SO_4 and read on a plate reader at 450 nm.

4.9 Scratch Score and Body Weight

The piglets were weighed and a scratch score of 0-3, as previously described by Widowski, 2003 recorded weekly for the duration of the 10 weeks of the experiment [19].

4.10 Statistical Analysis

All data were analysed using a mixed model in ASReml version 4.1. Any continuous data that were not normally distributed were transformed (either log or square root). All binary data were analysed using a generalised linear mixed model with the logit-link function, where the implicit residual variance on the underlying scale is $\pi^2/3$. Count data were analysed assuming a Poisson distribution or where there was over-dispersion a negative binomial regression.

The fixed effects fitted to the executive function and novel object data included replicate (1-4), parity (0-5), sex (F, M), sucker enrichment (barren or enriched), weaner enrichment (barren or enriched), and all significant ($P<0.05$) two-way interactions. To account for repeated measures on animals, animal ID was included as a random term. Sow ID was also fitted as a random term to separate the within and between litter variation. The same model was fitted to the maze data but the fixed effect of round (1-4) was also included.

Data for the open field/novel object test were analysed using a general linear model. Three analyses were conducted. Data for the open field test (first 3 min) were considered seperately to the data from the novel object test (last 2 min). Data were then analysed for the entire 5 min test period. Non-normally distributed data were natural-logarithmically transformed and when this occurred back transformed means are shown in parenthesis.

The immunological data were only collected on 1 replicate but across 4 time points, therefore the fixed effects of parity (0-5), sex (F, M), day, sucker enrichment (barren or enriched), weaner

enrichment (barren or enriched), and all significant ($P<0.05$) two-way interactions were included in the model. The random terms of sow and animal ID were also included.

The cortisol data were tested for normality using the Kolmogorov-Smirnov statistic and homogeneity of variance was tested using Levene's test. No transformations were necessary. Repeated measures analysis of variance (ANOVA) was used to compare the plasma concentrations of cortisol within and between groups. The within-subjects factors were time. The between-subjects factor was treatment.

5. Results

5.1 Open Field/Novel Object Test

The mean (±SEM) number of lines crossed during the novel object test was significantly greater for pigs that were provided with enrichment during the sucker phase than pigs that were housed in barren pens during the sucker phase (18.66 ±1.82 v 13.52 ±1.63, P<0.01, Figure. 5). The mean (±SEM) number of grunts produced during the novel object test was greater for pigs that were provided with enrichment during the sucker phase than pigs that were housed in barren pens during the sucker phase (16.46 ±1.43 v 11.30 ±1.33, P<0.01). The mean (±SEM) number of times that the pig investigated the novel object was significantly greater for pigs that were raised in barren pens in the weaner phase than pigs that were provided with enrichment in the weaner phase (6.12 ±0.48 v 4.40 ±0.46, P<0.01). There was no significant effect of enrichment on emergence time, number of grunts during the open field test, the number of lines crossed during the open field test, the time taken to interact with the novel object, number of squeals during the open field test or the novel object test, the number of urinations or the number of grunts during the open field test.

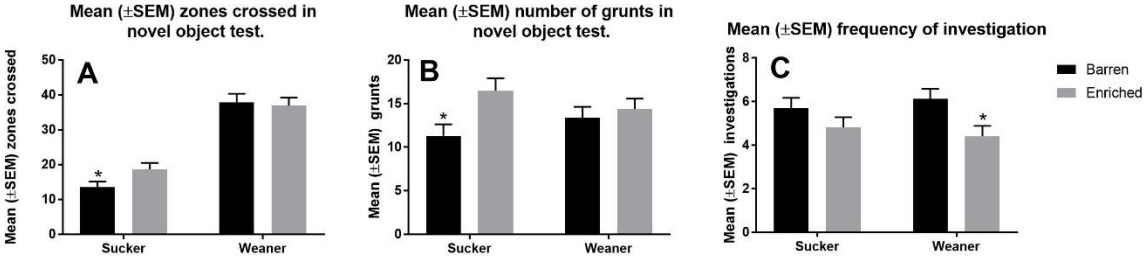

*Figure 5. Behavioural responses of pigs in the novel object test. Panel A shows the number of zones crossed, panel B shows the number of grunts and panel C shows the number of investigations of the novel object. * indicates a significant difference (P<0.05) between pigs provided with enrichment and pigs that were housed in a barren environment in each phase.*

5.2 Maze Test

In round one of the maze test the mean (±SEM) time taken for pigs to emerge from the start box was not significantly different between pigs that were provided with enrichment in the sucker phase and pigs that were housed in barren pens during the sucker phase (1.35 ±0.55 s v 1.37 ±0.51 s, P >0.05). In rounds two, three and four the mean (±SEM) time taken for pigs to emerge from the start box was significantly greater for pigs that were provided with enrichment during the sucker phase than pigs that were housed in barren environments during the sucker phase. Round 2; 2.65 ± 0.51 s v 1.13 ± 0.55 s, round 3; 2.3 ±0.51 s v 1.00 ± 0.55 s, round 4; 2.4 ± 0.51 v 1.02 ± 0.51 (p < 0.05). The mean (±SEM) total time (s) spent in all traps was significantly greater for pigs that were provided with enrichment in the sucker phase and housed in a barren pen in weaner phase (EB) than pigs that were provided with enrichment in the sucker phase and provided with enrichment in the weaner phase (EE), pigs that were housed in barren pens in the sucker phase and the weaner phase (BB) and pigs that were housed in barren pens during the sucker phase and provided with enrichment in the weaner phase (BE) (22.86 ±1.16 v 15.88 ±1.16 v 14.12 ±1.17 v 15.81 ±1.16, P<0.05). There was a trend (P=0.09) toward the mean (±SEM) time taken to reach the reward being greater for pigs that were provided with enrichment in the sucker phase than pigs that were housed in a barren environment

during the sucker phase (68.77 ±5.16 v 63.66 ±5.16, P=0.09 Figure 6). There was a trend (p = 0.057) toward the mean (±SEM) time spent in trap two being greater for pigs that were enriched in the sucker phase than pigs that were housed in barren pens in the sucker phase (11.76 ± 1.12 s v 8.51 ± 1.12, P=0.057). There was a trend (p=0.09) toward the mean (±SEM) time spent in trap 2 to be less for pigs that were provided with enrichment in the weaner phase than pigs that were housed in barren pens in the weaner phase (9.01±1.12 s v 11.11 ±1.12 s).

When the performance of all pigs in the maze test was analysed there were significant effects of round on the time taken to solve the maze, the percentage of pigs that got caught in trap one, the percentage of pigs that got caught in trap two, the number of times that the pigs got caught in a trap and the time spent in trap one and trap two over the four rounds (Figure 7). The mean (±SEM) s taken to solve the maze reduced from 148.39 ±6.13 s in round one to 61.00 ±6.11 s in round two, to 32.8 ±6.06 s in round three to 22.66 ±9.09 s in round four (P<0.001). The mean (±SEM) percentage of pigs that got caught in trap one reduced from 96 ±0.04 % in round one, to 90 ±0.04% in round two, to 71 ±0.04% in round three, to 48 ±0.04% in round four (P<0.001). The mean (±SEM) percentage of pigs that got caught in trap two in round one was 97 ±0.03% and was 97 ±0.03% in round two. This variable was reduced to 88 ±0.03% in round three and to 80 ±0.3% in round four (P<0.01). The mean (±SEM) number of times that pigs were caught in trap one reduced from 2.21 ±0.12 in round one, to 0.96 ±0.12 in round two, to 0.71 ±0.12 in round three, to 0.46 ±0.12 in round four (P<0.001). The mean (±SEM) number of times that pigs were caught in trap two reduced from 1.69 ±0.10 in round one, to 1.18 ±0.10 in round two, to 0.97 ±0.10 in round three, to 0.90 ±0.10 in round four (P<0.001). The mean (±SEM) total time that pigs spent in trap one reduced from 38.84 ±2.34 s in round one, to 10.85 ±2.34 s in round two, to 3.69 ±2.34 in round three, to 1.41 ±2.34 s in round four(P<0.001). The mean (±SEM) total time that pigs spent in trap two reduced from 42.69 ±1.13 s in round one, to 14.78 ±1.13 s in round two, to 5.69 ±1.13 in round three, to 2.79 ±1.14 s in round four (P<0.001).

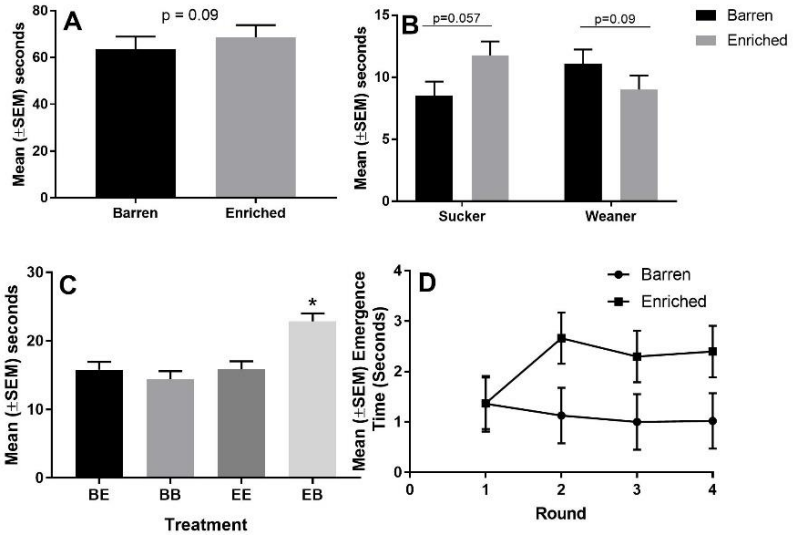

*Figure 6. The effect of enrichment on performance in the maze test. Panel A depicts the mean time taken to reach the reward, panel B depicts mean (±SEM) time spent in trap 2, panel C depicts total time spent in all traps and panel D depicts the time taken to emerge from the start box over the four rounds of the test. * indicates significant difference, P<0.05.*

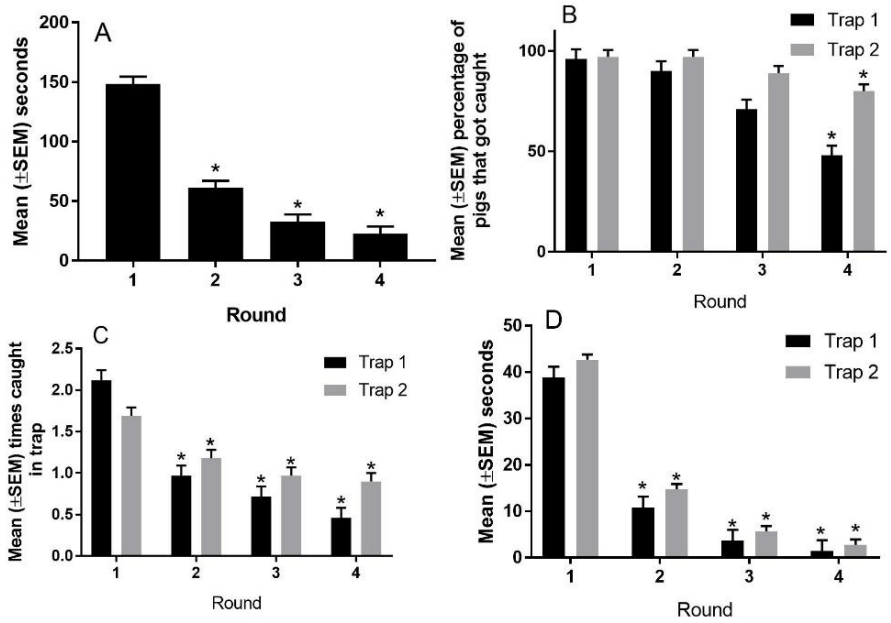

*Figure 7. The performance of pigs in the maze test over successive rounds. Panel A depicts time taken to solve the maze over four rounds for all pigs, panel B depicts the percentage of pigs that got caught in trap 1 or 2 over the four rounds for all pigs, panel C depicts the number of times a pig was caught in each trap for all pigs and panel D depicts the amount of time pigs spent in each trap for all pigs. * indicates a significant difference from round one (P<0.05).*

5.3 Executive Function Test

There was a trend (P=0.07) toward the mean (±SEM) proportion of pigs that reached the correct zone to be greater for pigs that were provided with enrichment in the weaner phase than pigs that were housed in barren pens in the weaner phase (84 ±0.08% v 66 ±0.116%, Figure 8). There were no other significant effects of enrichment. There was a significant (P<0.001) increase in the mean (±SEM) percentage of pigs that reached the correct zone on their first try from 17 ±0.058% in round one to 29% ±0.058 in round two to 41% ±0.058 in round three (Figure 8). There was a significant (P<0.001) decrease in the number of zones crossed to get to the correct zone from 2.16 ±0.083 zones crossed in round one to 1.88 ±0.083 zones crossed in round two to 1.75 ±0.083 zones crossed in round three (Figure 8). There was a significant (P<0.01) decrease in the mean (±SEM) time taken to reach the correct zone from 31.29 ±2.43 s in round one to 18.84 ±2.23 s in round two to 17.30 ±2.19 s in round three (Figure 8).

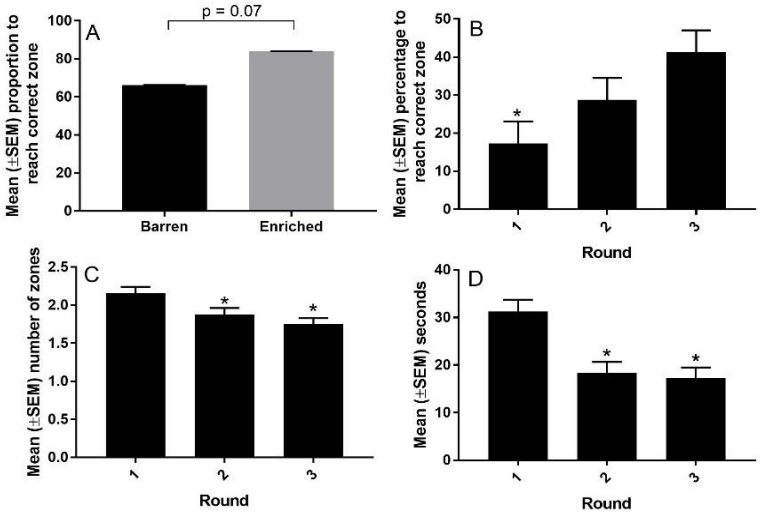

*Figure 8. The effect of enrichment and round on the performance of pigs in the executive function test. Panel A shows that proportion of pigs that reached the reward when enriched in the weaner phase. Panel B depicts that percentage of all pigs that reached the reward for rounds 1, 2 and 3. Panel C depicts the mean number of zones that the pigs crossed before they reached the reward for rounds 1, 2 and 3. Panel D depicts the time taken to reach the reward for all pigs in round 1, 2 and 3. *= significant difference from round 1, p<0.05.*

5.4 Cortisol Response to Novel Object/Open Field Test

There was no effect of treatment on the concentration of cortisol in plasma measured for 2h prior to the introduction into an open field/novel object test or for 2h after an open field/novel object test (Figure 9, P>0.05).

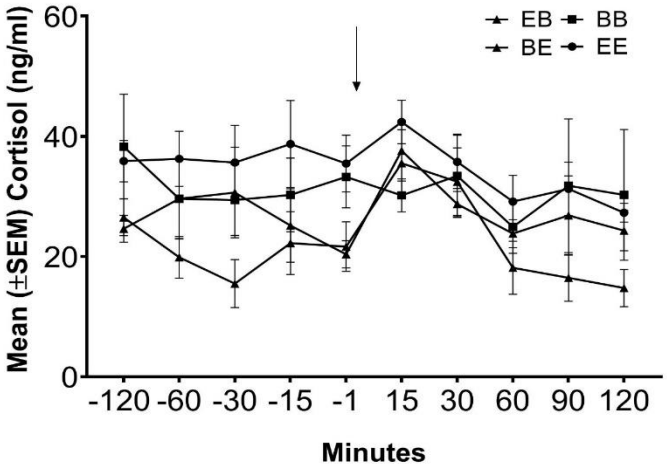

Figure 9. The mean (±SEM) change in cortisol before and after exposure to an open field test. The arrow depicts the time when the test was applied. Pigs were exposed to a 3 min open field test (indicated by the arrow). Blood samples were collected 120 min, 90 min, 60 min, 30 min, 15 min and 1 min prior to the test and then 15 min, 30 min, 60 min, 90 min and 120 min after the completion of the open field test.

5.5 Immune Function

5.5.1 Interleukin 10

The mean (±SEM) concentration of interleukin 10 (IL 10) in plasma for pigs that were provided with enrichment in the sucker phase and pigs that were housed in barren pens in the sucker phase 24 h before, 24 h after, 21 days after and 65 days after weaning is shown in Figure 10A. The mean (±SEM) concentration of IL 10 was significantly greater in pigs that were housed in a barren pen during the sucker phase than pigs that were provided with enrichment in the sucker phase 24 h after weaning and 65 days after weaning ($P<0.05$). The mean (±SEM) concentration of interleukin 10 was not significantly different between pigs that were housed in a barren pen during the sucker phase and pigs that were provided with enrichment in the sucker phase 24 h prior to weaning and 21 days after weaning ($P>0.05$).

The mean (±SEM) concentration of IL 10 in plasma was significantly lower in pigs that were housed in a barren pen during the weaner phase than pigs that were provided with enrichment in the weaner phase 24 h after weaning and 65 days after weaning ($P<0.05$; Figure 10B). The mean (±SEM) concentration of IL 10 was significantly greater for pigs that were housed in a barren environment than pigs that were provided with enrichment in the weaner phase 24h prior to weaning ($P<0.05$). The mean (±SEM) concentration of interleukin 10 was not significantly different between pigs that were housed in a barren pen during the weaner phase and pigs that were provided with enrichment in the weaner phase 21 days after weaning ($P>0.05$).

5.5.2 Tumor Necrosis Factor Alpha

The mean (±SEM) concentration of tumor necrosis factor α (TNFα) in plasma was significantly greater in pigs that were housed in a barren pen during the sucker phase than in pigs that were provided with enrichment in the sucker phase 24 h after weaning ($P<0.05$; Figure 10C). The mean (±SEM) concentration of TNFα was not significantly different between pigs that were housed in a barren pen during the sucker phase and pigs that were provided with enrichment in the sucker phase 24 h prior to weaning, 21 days after weaning or 65 days after weaning ($P>0.05$).

The mean (±SEM) concentration of TNFα in plasma was significantly greater in pigs that were housed in a barren pen during the weaner phase than for pigs that were provided with enrichment in the weaner phase 24 h after weaning ($P<0.05$; Figure 10D). The mean (±SEM) concentration of TNFα was not significantly different between pigs that were housed in a barren pen during the weaner phase and pigs that were provided with enrichment in the weaner phase, 21 days after weaning or 65 days after weaning ($P>0.05$). The mean (±SEM) concentration of TNFα for pigs that were provided with enrichment in the weaner phase was greater than pigs that were housed in barren pens 24h prior to weaning ($P>0.05$).

5.5.3 Interferon gamma

The mean (±SEM) concentration of interferon gamma (IFγ) in plasma was not significantly different between pigs that were housed in a barren pen during the sucker phase and pigs that were provided with enrichment in the sucker phase 24 h prior to weaning, 24h after weaning, 21 days after weaning or 65 days after weaning ($P>0.05$; Figure 10E).

The mean (±SEM) concentration of IFɣ in plasma was significantly greater in pigs that were provided with enrichment in the weaner phase 24 h after weaning than pigs housed in a barren pen ($P<0.05$). The mean (±SEM) concentration of IFɣ was not significantly different between pigs that were provided with enrichment and pig that were housed in a barren pen during the weaner phase 24 h before or 21 days after weaning ($P>0.05$). The mean (±SEM) concentration of IFɣ was significantly lower in pigs that were provided with enrichment in the weaner phase 65 d after weaning than for pigs housed in a barren pen ($P<0.05$).

5.5.4 Interleukin 6

The mean (±SEM) concentration of interleukin 6 (IL6) in plasma was significantly greater in pigs provided with enrichment in the sucker phase 24 h after weaning, and 21 d after weaning than pigs housed in a barren pen during the sucker phase ($P<0.05$; Figure 10G). The mean (±SEM) concentration of IL6 was not significantly different between pigs provided with enrichment in the sucker phase and pigs housed in a barren pen during the sucker phase 24 h prior to weaning and 65 days after weaning ($P>0.05$).

The mean (±SEM) concentration of IL6 in plasma was significantly greater in pigs that were provided with enrichment in the weaner phase 24 h prior to weaning and 24 h after weaning than pigs housed in a barren pen during the weaner phase ($P<0.05$; Figure 10H). The mean (±SEM) concentration of IL6 was not significantly different between pigs that were housed in a barren pen during the weaner phase than pigs that were provided with enrichment in the weaner phase, 21 days after weaning or 65 days after weaning ($P>0.05$).

The change in cytokines when the data were analysed incorporating all enrichment treatments is shown in Figure 11. The mean (±SEM) concentration of IL10 was greater 24 h after weaning for pigs in the BE group than pigs in the EE, BB or EB groups ($P<0.05$; Figure 11A). The mean (±SEM) concentration of IFɣ was greater 24 h after weaning for pigs from the BE group than pigs from the EE, BB or EB groups ($P<0.05$; Figure 11B). The mean (±SEM) concentration of TNFα was greater 24 h after weaning for pigs in the BB group than the EB or the BE groups ($P<0.05$; Figure 11C). The mean (±SEM) concentration of TNFα was lower 24 h after weaning for the EB group than for the EE, BB or BE groups ($P<0.05$; Figure 11D).

5.6 Cell count

The mean (±SEM) counts for total numbers of immune cells and the proportion of cell types are shown in Figure 12. There was no significant difference in the number of white blood cells, the percentage of white blood cells that were neutrophils, the number of neutrophils, the number of lymphocytes, the percentage of white blood cells that were lymphocytes, the number of monocytes, the haemoglobin concentration, haematocrit, mean corpuscular volume, mean corpuscular haemoglobin or concentration between pigs that were provided with enrichment in the sucker phase and pigs housed in barren pens during the sucker phase or between pigs that were provided with enrichment during the weaner phase or pigs that were housed in barren environments during the sucker phase.

There was an overall effect of enrichment in the weaner phase on the number of platelets with pigs housed in barren pens having a greater number of platelets than pigs provided with enrichment during the weaner phase ($P<0.05$, Figure 12). There was an overall effect of treatment when all times were combined and analysed together for haemoglobin, haematocrit, red blood cell distribution width and the number of platelets ($P<0.05$, Figure 13). The mean (±SEM) concentration of haemoglobin was

significantly lower for BB pigs than for BE, EB or EE pigs (P<0.05, Figure 13A). The mean (±SEM) haematocrit was lower for BB pigs than for BE, EB or EE pigs (P<0.05, Figure 13B). The mean (±SEM) red blood cell distribution width for BB pigs was significantly greater than BE pigs and EE pigs (P<0.05, Figure 13C) but was not significantly different from EB pigs (P>0.05, Figure 13C).

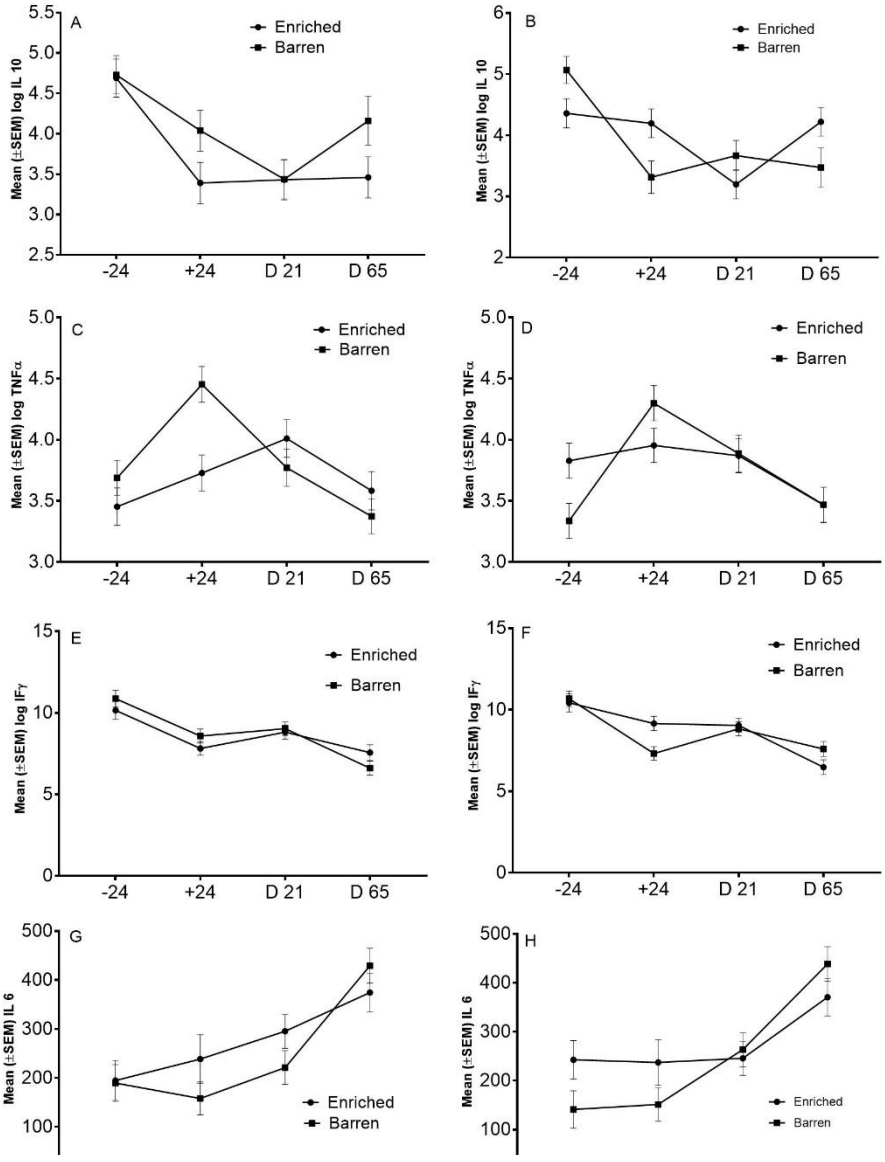

Figure 10. The cytokine response for Interleukin 10 (IL10), tumour necrosis factor α (TNFα), interferon γ (IFγ) and interleukin 6 (IL6) 24 h prior to weaning (-24), 24 h after weaning (+24), 21 days after weaning (D21) and 65 days after weaning (D65) when analysed as enrichment provided in the sucker or weaner phase. Panel A depicts the change in IL 10 when enrichment was provided in the sucker phase and panel B depicts the change in IL10 when enrichment was provided in the weaner phase. Panel C depicts the change in TNFα when enrichment was provided in the sucker phase and panel B depicts the change in TNFα when enrichment was provided in the weaner phase. Panel D depicts the change in IFγ when enrichment was provided in the sucker phase and panel E depicts the change in IFγ when enrichment was provided in the weaner phase. Panel G depicts the change in IL6 when enrichment was provided in the sucker phase and panel E depicts the change in IL6 when enrichment was provided in the weaner phase.

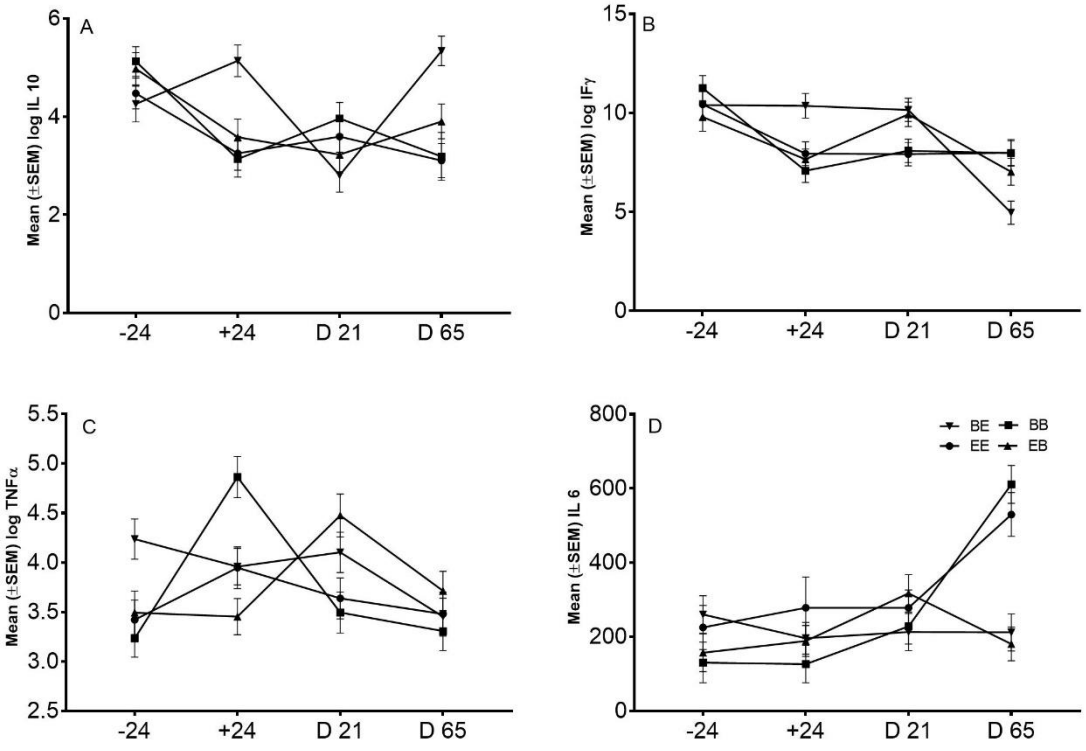

Figure 11. The cytokine response for Interleukin 10 (IL10), tumour necrosis factor α (TNFα), interferon γ (IFγ) and interleukin 6 (IL6) 24 h prior to weaning (-24), 24 h after weaning (+24), 21 days after weaning (D21) and 65 days after weaning (D65) when all combinations of enrichment were analysed. Panel A depicts the change in IL10, panel B depicts the change in IFγ, panel C depict the change in TNFα and panel D depicts the change in IL 6.

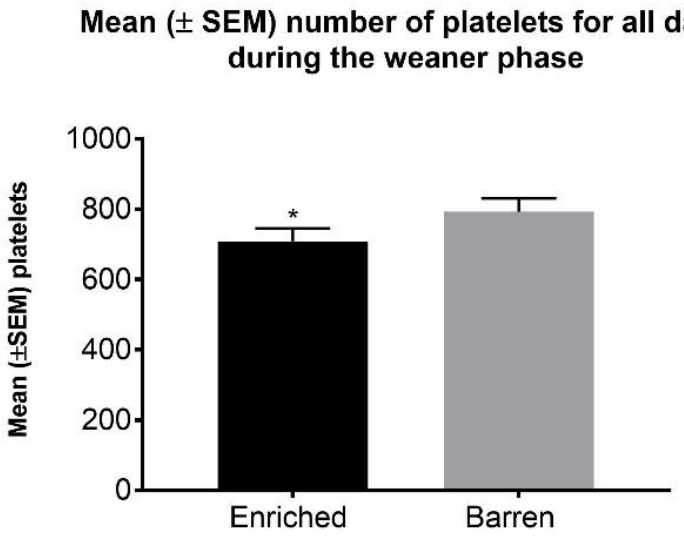

*Figure 12. The effect of enrichment in the weaner phase on the number of platelets in blood when all days are combined. * indicates a significant difference. P<0.05*

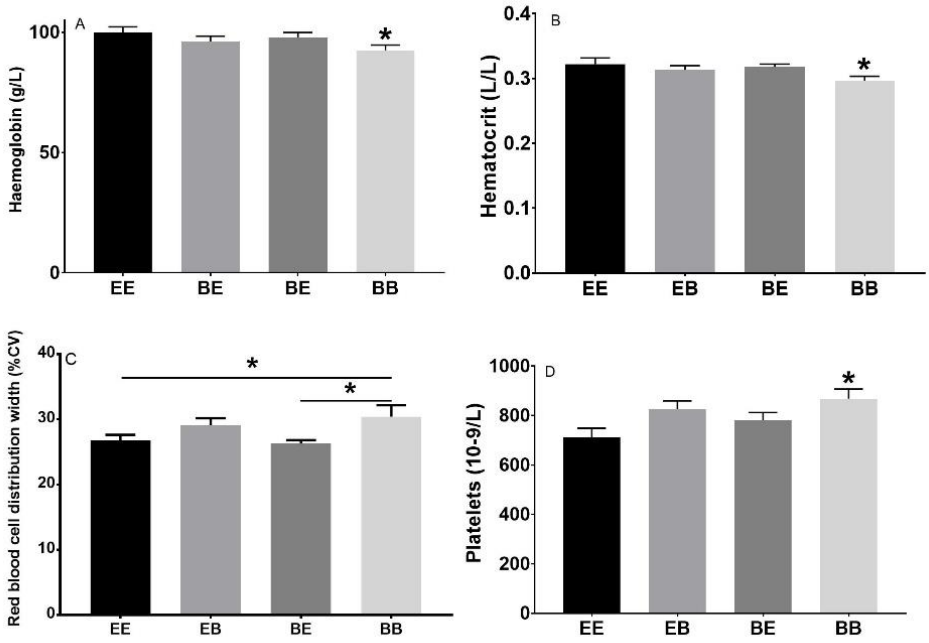

*Figure 13. The overall effect of treatment on red blood cell parameters. Panel A depicts haemoglobin, panel B depicts haematocrit, panel C depicts red blood cell distribution width and panel C the number of platelets. Data from all times were combined for each treatment. Panel A, panel B and panel D * indicates significant (P<0.05) difference from the EE group. Panel C differences between groups are indicated with line and *.*

5.7 Scratch score and body weight

There were no significant differences in body weight between the treatments at any time (P>0.05; Figure 15). There was significantly less scratches on pigs from the EE group 7 d after weaning than the other groups (P<0.05) and significantly more scratches on the pigs in the EB group that the other groups 14 d after weaning, (P<0.05; Figure 14).

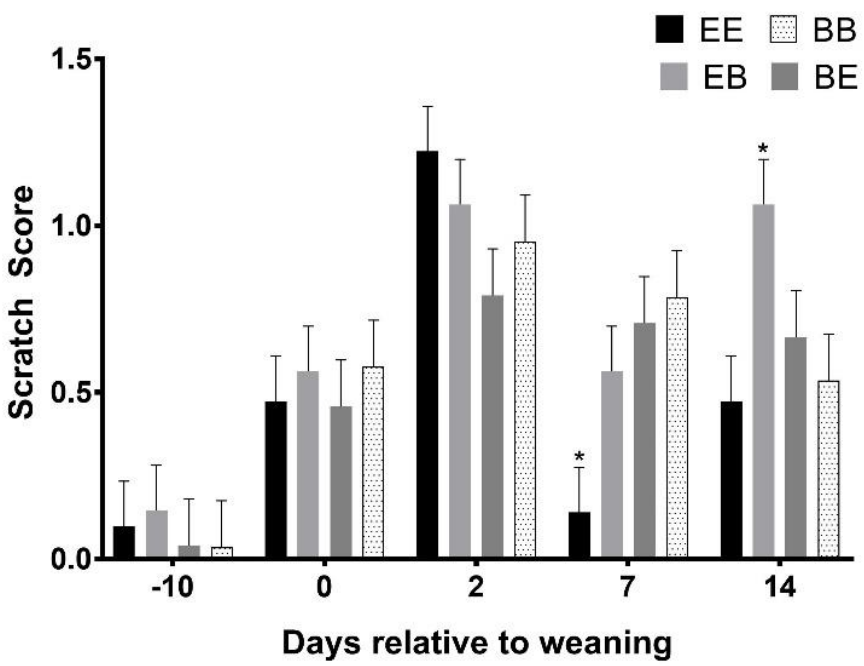

*Figure 14. The effect of the provision of enrichment on the number of scratches recorded on the pigs. Day 0 indicates the day of weaning, other days are relative to weaning. * indicates a significant difference between the groups, P<0.05.*

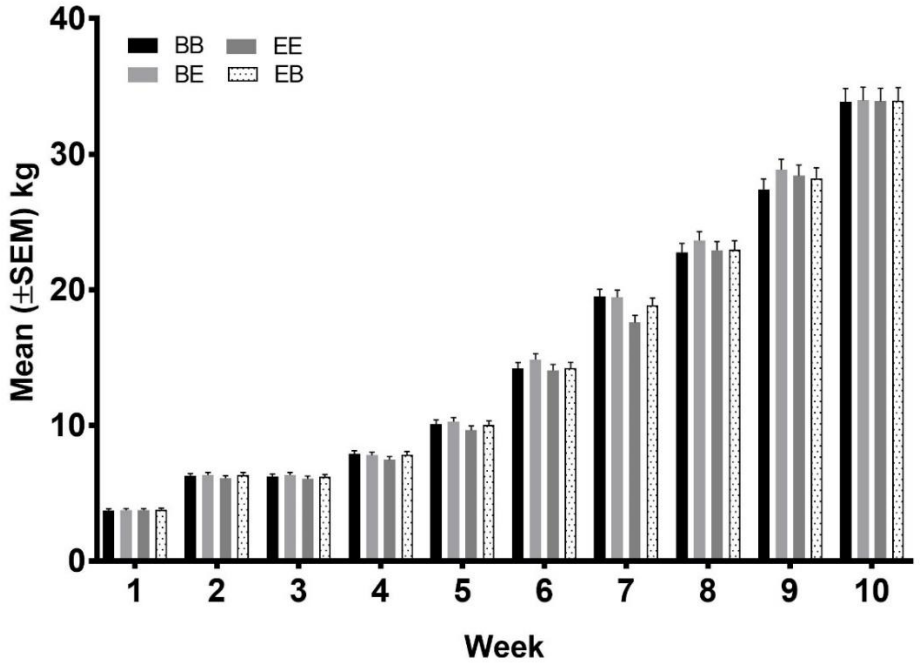

*Figure 15. The effect of the provision of enrichment on pig weight to 10wks of age. Pigs were weighed weekly from week 1 to week 10. * indicates a significant difference between the groups, P<0.05.*

7. Discussion

Our data suggests that the provision of enrichment blocks affected the behaviour of pigs in a maze test, in an open field/novel object test and in an executive function test. The performance of the pigs in the maze test and in the executive function test improved significantly with each exposure to the test and this a good indication that the pigs were able to learn the test. The reduced number of scratches on pigs that were enriched in both the sucker and the weaner phase 7 days after weaning suggests that there were possibly effects on aggression and fighting and that the provision of enrichment may have reduced the number or severity of the fights. The greater number of scratches on pigs that were enriched in the sucker phase but not in the weaner phase 14 days after weaning suggests there may have been more fights or more aggressive encounters when pigs were first provided with enrichment and then the enrichment was taken away. In addition, we have evidence that at least some aspects of the immune system of the pigs were affected by the addition of the enrichment blocks. Combined, these data indicate that there were several effects of the provision of enrichment blocks and these affects are likely to influence the welfare of pigs.

The benefits of the provision of enrichment has been evaluated in a number of ways, for example by evaluating glucocorticoids, by evaluating the immune system, by evaluating heart rate variability and agonistic interactions between animals or by evaluating changes in the brain [8, 13, 14]. Our hypothesis was that the benefit of enrichment is not related to the number of interactions that the animals has with the enrichment but whether the provision of enrichment can influence the behaviour of the pigs when tested outside of their home pen. Our data supports this hypothesis and we have evidence that the provision of enrichment to pigs in the sucker phase increased their willingness to interact with their environment and provision of enrichment in the weaner phase may have improved the performance of pigs in the executive function test. Oro-nasal contact with the blocks was relatively infrequent before pigs were about 25 days old and there was a less than 10% probability that the pigs would interact with the blocks at this age. Nonetheless, our data indicate that the presence of the enrichment blocks during the sucker phase altered the performance of the pigs in the behavioural tests. An important aspect of environmental enrichment is the opportunity for animals to apply a variety of cognitive processes to solve problems [4] and environments that foster flexible behavioural repertoires are more effective than environments that foster uniformity or are barren [15]. The data from the current experiment suggests that a more complex environment in the sucker phase had benefits for pigs and the benefit is not determined by the number of interactions the pig has with the enrichment. More likely, the benefit for the pig are that the addition of the blocks provides opportunity for pigs in the sucker phase to solve problems, albeit it the simple problem of how to navigate around or over an enrichment block, and present a more complex environment that facilitates the need for a greater behavioural repertoire.

Enrichment in the weaner phase may have assisted in improving the learning ability of the pigs. The proportion of pigs that reached the correct zone in the executive function test was greater and time spent in trap 2 was less for pigs provided with enrichment during the weaner phase than for pigs housed in barren pens during the weaner phase. Similarly, rats housed in enriched environments made less errors in a maze test and displayed greater working memory than rats housed in barren environments [16]. There were accompanying functional changes to the brain of these rats and this indicates that the rats housed in the enriched environment had functional differences in the way their brain had developed [16]. There is little evidence of this in pigs and we present the first, although not conclusive, evidence that enrichment can alter cognitive function in young pigs. It is important to note

that the previous research in this area has used complex enriched environments. The rats housed in enriched pens in the aforementioned research had access to deep litter, toys, running wheels and an overall more complex environment. The pigs in the current study were provided only with enrichment blocks. Therefore, it is not unexpected that evidence of improved cognition was seen, albeit less compelling and to a lesser extent than previous research in other species. Our results, may simply reflect the relative simplicity of the enrichment used in the current experiment.

We have good evidence that pigs younger than 10 weeks of age can learn quickly and learn complex tasks quickly. When all pigs were combined and analysed based on their performance in successive exposures to the maze test and the executive function test their performance significantly improved over time. For example, the time taken for pigs to solve the maze reduced from 148 s in round 1 to 22 s in round 4. The number of animals that reached the zone with the reward the first try in the executive function test significantly increased from 17% in the first test to 41% in the third test and the number of zones crossed to reach the reward and the mean time to reach the reward both significantly decreased from test one to test three. Combined, this indicates that the pigs have the ability to learn complex tasks at this age. In particular, the improved performance in the executive function test suggests the pigs learned to differentiate between two audible cues and associate one of those cues with a reward. This is a complex task and the ability of the pigs to learn this task has implications for management practices and housing systems applied at this age. It reaffirms that the sucker phase and the weaner phase are important developmental stages for pigs and their experiences during this time can shape their behaviour through life.

The provision of enrichment can alter the function of the immune system in mice, rats, goats and pigs [8, 12, 17]. We investigated the effect of the provision of enrichment on total cell numbers and on four cytokines, IL-10, TNFα, IF-ɣ and IL-6. We hypothesised that the provision of enrichment would attenuate the inflammatory response to weaning. There was a significant attenuation in the TNFα response to weaning for pigs that were provided with enrichment in the sucker or weaner phase 24h after weaning compared to pigs that were housed in barren pens in the sucker or weaner phase. There was not a significant increase in TNFα 24 h after weaning for pigs that were provided with enrichment whereas there was a significant increase in TNFα for pigs that were housed in a barren pen. This is evidence that there was an attenuated pro-inflammatory response to the weaning event for pigs that that were provided with enrichment either in the sucker or weaner phase. This suggests that the provision of enrichment in some way enabled the pigs to cope more efficiently with the stressors associated with weaning, at least in terms of their inflammatory response to weaning. The difference in platelet concentration, variation in red blood cell width, haemoglobin, and haematocrit in the BB treatment group are consistent with an attenuated inflammatory status. These data support the notion that the pigs raised in a barren environment may have had a heightened inflammatory status when compared to pigs raised in an enriched environment. This is evidence that provision of enrichment blocks can influence the immune function of sucker and weaner pigs. The implications of this for the long term welfare and productivity of sucker and weaner pigs requires further investigation.

The implication of this for the welfare of the animal cannot be clearly determined from the current research, however, our data support our hypothesis and indicate that the provision of enrichment has benefits for the function of the immune system and may alter the inflammatory response to weaning. This may have long term benefits and enable pigs to cope better with immune challenge and be more resilient when faced with stressors later in life.

While the effect of enrichment on the response of TNFα to weaning was clear the response of IL-10, IF-ɣ and IL-6 were less clear. IL-10 is an anti-inflammatory cytokine and was greater 24h after weaning in pigs that were provided with enrichment in the weaner phase than pigs that were housed in barren pens in the weaner phase. This is in keeping with the anti-inflammatory role of IL-10 and with the attenuated TNFα response to weaning. A greater concentration of IL-10 generally results in a lower concentration of pro-inflammatory cytokines like TNFα. There was, however, a greater concentration of IL-10 24h after weaning for pigs that were housed in barren pens in the sucker phase than provided with enrichment in the sucker phase. Interleukin 6 can increase due to inflammation caused by a pathogen or can increase due to muscular exertion in response to increased muscular activity or exercise [18]. Our data indicate that there was an overall increase in the concentration of IL-6 over time and that pigs provided with enrichment had greater IL-6 24h after weaning than pigs housed in barren pens. This could be due to a pro-inflammatory response or due to increased activity in the pigs that were provided with enrichment. There was no increase in IL-6 from the pre-weaning concentration to 24 h after weaning for either group and this indicates that IL-6 production was not increased in response to weaning per se. It is not possible to determine if the difference in IL-6 we have detected was due to increased activity, however, it seems unlikely that it was due to a difference in coping between the two groups as there was not an increase in IL-6 24 h after weaning for either group. IL-6 did increase for both groups by 65 d post weaning and this was expected as the concentration of IL-6 increases with age as the immune system matures.

8. Implications & Recommendations

The current project has identified that enrichment in the sucker and weaner phase can affect the behaviour of pigs, their ability to learn and affect their immune system and the immune response to weaning. Our data support the notion that the benefits of enrichment cannot be accurately gauged by measuring the interactions the animal has with the enrichments and it may simply be beneficial to live in a more complex environment. Although this project has not identified one clear benefit of the provision of the enrichment blocks it has identified that enrichment provided in the sucker phase does have benefits and that enrichment provided in the weaner phase does have benefits. In addition, pigs in the weaner phase can learn complex tasks. The overall implication of this research is that environmental enrichment likely impacts the behaviour, learning ability and immune function of young pigs. The current experiment could not identify what the longer term implications of that may be, however, we speculate that the pigs provided with enrichment would be better prepared to cope with challenge and may adapt faster to new environments. We have highlighted that the early rearing environment is important and that the management and husbandry at an early age can have long term implications for pigs. The enrichment we used in this study was very simple, an enrichment block, and we have been able to show a number of effects on the pigs. Even the simplest of enrichments may be important and that there is merit in developing more enrichment devices that are suitable for use in the Australian pig industry.

9. Intellectual Property

The intellectual property generated from the report lies in the effectiveness of the enrichment blocks in benefiting the welfare of pigs in the sucker and weaner phases. The intellectual property pertaining to the content and make-up of the blocks is owned by Ridley's as the information presented in this report pertains to the effectiveness of the enrichment block.

10. Literature cited

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11. Publications Arising

The following were submitted for publication to the Australasian Pig Science Association for inclusion in Manipulating Pig Production XVI a special edition of Animal Production Science.

Sucker and weaner pigs prefer brick shaped enrichment blocks over cube or wedge shaped enrichment blocks. *C. R. Ralph, J. A. Winfield, G. F. Macnamara, B. L. F. Macnamara, C. O'Shea, E. J. S. Hall and G. M. Cronin*

Provision of enrichment blocks alters red blood cell parameters in sucker and weaner pigs. *S. A. Barnes, S. Kitessa, G. Cronin, and C.R. Ralph*

The effects of provided enrichment in the sucker phase on piglet behaviour post-weaning *L. A. McKenny, G.C. Cronin, M. Hebart, Plush K.J. and C.R. Ralph*

Efficiency to complete the maze test is decreased in young pigs enriched during the sucker phase *J. Zemitis, G. M. Cronin, M.L. Hebart and C. R. Ralph*