Body composition and physiological changes associated with immunization of pigs against gonadotrophin releasing factor (GnRF) at two different live weights 3A-101

Report prepared for the Co-operative Research Centre for High Integrity Australian Pork

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Executive Summary

Boar taint is still an issue for Australian consumers and immunisation against GnRF (using the Improvac® vaccine) is an effective way to eliminate it. However, immunocastration may be associated with an increase in backfat and feed intake. In order to identify management strategies to address these issues it is necessary to further understand the physiological and nutritional changes which occur following the second vaccination of Improvac®. Therefore, the aim of this project was to identify and compare the physiological, nutritional and pork quality changes that occur following the second injection of the immunocastration vaccine, Improvac®, in light and heavy weight pigs. The hypotheses were:

1. Light weight pigs immunised against GnRF will have a lower fat to lean muscle ratio over time compared to pigs immunised at a heavier weight.
2. Pigs that receive feed ad libitum will have an improved growth rate and increased body fat composition compared to pigs that have a controlled feed intake.
3. Pigs immunised against GnRF will have improved sensory pork quality compared to entire males at both light and heavy slaughter weights.

A total of 64 Large White × Landrace × Duroc entire male and immunised male pigs were used in this experiment. The experiment was a 2×2×2 factorial with the main treatments being:

i) sex (entire males and immunised males);
ii) starting weight (50 kg (light) and 80 kg (heavy) LW) and;
iii) feeding regime (2.5 times maintenance (restricted) and *ad libitum*).

Growth performance, body composition and objective and sensory pork quality were determined.

There was no difference in growth performance between entire males and immunised males. The majority of fat deposition by immunised males occurred 2 to 3 weeks after the second dose of Improvac®. However, this only occurred when pigs received the second dose of Improvac® at heavier LWs (80 kg). When pigs were immunised at lighter LWs (50 kg) and/or on a restricted diet they had a reduced propensity to deposit fat, however, the restriction in feed intake adversely impacted on growth rate. Gender did not influence objective or sensory quality in this experiment. However, fail rate for quality grade (20.7% IM vs 29.8% EM) and re-purchase intention (26.7% IM vs 38.7% EM) was significantly lower for pork from immunised males compared to entire males across all treatment combinations.

Further research is required to address the increase in backfat and decrease in lean deposition of immunised males at heavier liveweights. This may include strategies such as feeding the appropriate lysine level, using pST for 2 weeks only and feeding a low energy diet.

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1. Introduction

Payment systems in Australia are based on carcase weight and carcase fatness with producers penalised for carcases with increased backfat. Entire males are preferred by producers as they have better feed conversion and less subcutaneous fat compared to physically castrated pigs, however the occurrence of boar taint which can reduce eating quality is an issue. Immunisation of pigs against gonadotrophin releasing factor (GnRF) with the Improvac® vaccine is an alternative to physical castration and consists of two doses, one administered at approximately 9-10 weeks of age as a priming dose and the other typically administered at 4-5 weeks prior to slaughter to activate the response. This allows the pig to grow as an entire boar with the associated positive effects on growth and carcase leanness. However, after the second vaccination there is an increase in feed intake and backfat (Dunshea *et al*. 2001; Cronin *et al*. 2003; Oliver *et al*. 2003).

With increased pressure on welfare grounds to stop the practice of physical castration without the use of anaesthetics, it is likely that the proportion of pigs to be immunised against GnRF will increase exponentially in many of the 60 countries where Improvac® is registered. To date most research with Improvac® has concentrated on getting the product registered with little attention paid to either nutrient requirements or impact on body composition. For example, the nutrient requirements have been assumed to be the same as that for female pigs of the same genotype. However, Moore *et al*. (2011) have determined that the lysine requirements of immunised males are closer to that of entire males than we have assumed and this could have important consequences to changes in carcase composition following the second vaccination.

While it is clear Improvac® is an effective product to control boar taint and hence a strategy to improve meat quality, the increase in fat deposition associated with an increase in feed intake is a reason why some producers do not use the technology because of the price penalties on overfat carcases. If we are able to understand the physiological and nutritional changes that occur following the second vaccination, and importantly the timing of these changes, then we would be in a better position to identify management strategies that could improve carcase quality and eating quality of pork, without any major effect on the cost of production. The increase in feed intake after the second vaccination is due to a reduction in testicular hormones (Bauer *et al*. 2009). This in turn can lead to increased growth and backfat. To better understand the physiological effects effect Improvac® is having, it is necessary to control feed intake to negate the increase in feed intake on body composition.

Recently in Australia there has been a market driven reduction in the slaughter weight of many pigs destined for the fresh pork market. Unfortunately there is a belief amongst some producers, processors and retailers that if male pigs are slaughtered at less than 90 kg live weight (LW) then there is no need for either physical castration or immunocastration because the incidence of boar taint is negligible. However, a recent survey (D'Souza *et al*. 2011) clearly showed a high proportion of pork samples exceeded the threshold level for androstenone (1 mg/g) and skatole (0.02 mg/g) regardless of slaughter weight (73 to 115 kg LW). D'Souza *et al*. (2004) have also shown that the deposition of fat between the different depots changes with LW, with the fat to muscle ratio increasing over time, hence any study of body composition changes following immunisation against GnRF needs to take initial LW into account.

Most research related to the eating quality of immunised males has concentrated on the incidence of boar taint rather than other aspects of eating quality. While boar taint is perhaps the most important factor that could affect the purchasing behaviour of consumers, there are other aspects of eating quality or taste that have not yet been considered. In examining the relationship between slaughter weight, nutrient requirements and changes in body composition following the second vaccination it is important to build in a component to assess eating quality so that the results of this research can be incorporated into pork quality predictive models, as well as being used to influence the actions of those in the industry who do not consider boar taint to be an issue.

The aim of this experiment was to identify and compare the physiological, nutritional and pork quality changes that occur following the second injection of the immunocastration vaccine, Improvac®, in light and heavy weight pigs. The hypotheses were:

1. Light weight pigs immunised against GnRF will have a lower fat to lean muscle ratio over time compared to pigs immunised at a heavier weight.
2. Pigs that receive feed ad libitum will have an improved growth rate and increased body fat composition compared to pigs that have a controlled feed intake.
3. Pigs immunised against GnRF will have improved sensory pork quality compared to entire males at both light and heavy slaughter weights.

2. Methodology

The experiment was conducted at the Department of Agriculture and Food Western Australia's (DAFWA) Medina Research Centre. The experimental protocol used was approved by the DAFWA Animal Research Committee and by the Animal Ethics Committee (2-12-11). The animals were handled according to the Australian code of practice for the care and use of animals for scientific purposes (NHMRC, 2004).

Animals and Experimental Design

A total of 64 Large White × Landrace × Duroc entire male and immunised male pigs were used in this experiment. The experiment was a 2×2×2 factorial with the main treatments being:

i) sex (S, entire males (E) and immunised males (I));
ii) starting weight (W, 50 kg (light) and 80 kg (heavy) LW) and;
iii) feeding regime (F, 2.5 times maintenance (restricted) and *ad libitum*).

Allocation and housing

On Day -26 (where Day 0 is the commencement of the experimental diets), 64 pigs of 26±3 kg liveweight (LW) and 32 pigs at 51±3 kg LW were sourced from a high health status commercial herd whose bloodlines were from the Pig Improvement Company. Due to housing constraints the experiment was conducted in two replicates of 32 from November 2012 to February 2013. Upon arrival the pigs were individually identified with ear tags, weighed and stratified on their LW with half the pigs from each weight receiving the priming dose of Improvac® (Pfizer Animal Health, Parkville, Australia). The pigs were group housed in a naturally ventilated grower shed. They had *ad libitum* access to a commercial feed via a single spaced feeder and water.

On Day -7 the pigs in each weight and priming dose group were stratified on LW and randomly allocated to a feeding regime. The pigs were then transferred to individual pens with a space allowance of 2.52 m^2 in a naturally ventilated shed. Each pig had access to water via a nipple drinker.

Diets and feeding regime

On Day 0 all pigs received the experimental diet and the second dose of the Improvac® vaccine was given to the pigs who had received the priming dose of Improvac®. Although the pigs were different weights and sexes and therefore had different requirements, all pigs received the same diet formulated to meet the requirements of entire males at 50 kg LW as determined from Moore *et al*. (2012) and Pork CRC project 2A-109. This ensured that the requirements of all the pigs were adequately met. The composition of the experimental diet is given in Table 1. Feed samples were analysed for quantitative amino acid composition (Animal Health Laboratories, Department of Agriculture and Food Western Australia, South Perth, WA, Australia) and the results are given in Table 2. Maintenance requirements for those receiving the Restricted treatment were determined weekly using the following equation E_m (kJ/d) = 444 kJ x $W^{0.75}$, where E_m = energy maintenance and W = liveweight.

Table 1 - The composition of the experimental diet.

Ingredients	(g/kg)
Barley	573
Wheat	100
Lupins	125
Canola meal	150
Meat meal	12.5
Tallow	25.0
Limestone	10.0
Salt	2.00
L-Lysine HCL	1.71
Methionine	0.26
Phyzyme	0.20
Vitamins and minerals[a]	0.70
Nutrient Composition[b]	
DE (MJ/kg)	13.5
Crude protein (%)	17.9
g SID Lys/MJ DE[c]	0.59

[a] Each kilogram of vitamin and mineral premix contains 7 MIU Vitamin A, 1.4 MIU Vitamin D_3, 20 g Vitamin E, 1 g Vitamin K, 1 g Vitamin B_1, 3 g Vitamin B_2, 1.5 g Vitamin B_6, 15 mg Vitamin B_{12} 12 g niacin, 10 mg pantothentic acid, 0.19 g folic acid, 30 mg biotin, 10.6 g Calcium pantothenatic, 60 g iron, 100 g zinc, 40 g manganese, 10 g copper, 0.2 g cobalt, 0.5 g iodine, 0.3 g selenium, and 20 g antioxidant.
[b] Calculated composition.
[c] SID: standardised ileal digestible lysine/MJ digestible energy.

Table 2 -Quantitative amino acid analysis of the diets used in the two replicates.

Amino acid (g/kg)	Replicate 1	Replicate 2
Histidine	3.60	3.33
Serine	8.40	7.80
Arginine	11.5	11.2
Glycine	8.90	9.50
Aspartic acid	14.9	14.5
Glutamic acid	37.0	33.3
Threonine	7.40	7.40
Cysteine-X	3.70	3.60
Alanine	7.70	8.00
Proline	15.7	17.2
Lysine	9.50	8.50
Tyrosine	5.60	5.20
Methionine	3.40	3.50
Valine	7.40	7.20
Isoleucine	6.40	6.00
Leucine	11.7	12.3
Phenylalanine	7.60	7.10

Slaughter procedure

On Day 28 the pigs were individually tattooed, removed from feed overnight and the following morning transported to a commercial abattoir (approx. 90 minute transport time). The pigs were stunned using a carbon dioxide, dip-lift stunner set at 85% CO_2 for 1.8 minutes (Butina, Denmark). Exsanguination, scalding, dehairing and evisceration were performed using standard commercial procedures.

Growth performance and carcass composition assessment

Pigs were weighed and feed refusal recorded on Days 0, 3, 7, 10, 14, 21 and 28 to determine average daily gain (ADG) and voluntary feed intake (VFI). Feed:gain was determined on a weekly basis from when the feeding of the experimental diets commenced. Ultrasound back fat depth, 65 mm from the dorsal midline at the point of the last rib, was measured on Day 0, 14 and 28 using a real time ultrasound (Renco Lean-Meter). Hot carcase weight (AUSMEAT Trim 13; head off, fore trotters off, hind trotters on; AUS-MEAT Ltd, South Brisbane, Qld, Australia) and P2 backfat depth, 65 mm from the dorsal midline at the point of the last rib (PorkScan Pty Ltd, Canberra, Australia) were measured approximately 35 minutes after exsanguination, prior to chiller entry (2^{o}C, airspeed 4 m/second).

Dual-energy x-ray absorptiometry (DXA) analysis

The pigs were scanned on Day 0, 14 and 28 using dual-energy x-ray absorptiometry. The pigs were removed from feed and fasted for approximately 16 hours before scanning. Immediately prior to scanning the pigs were weighed and then transferred to the DXA facility. They were injected intramuscularly with Stresnil® (azaperone 40 mg/mL, Stresnil Neuroleptic Injection for Pigs, Ausrichter Pty Ltd, NSW) at 2 mL/10 kg. When sufficiently sedated the pigs were transferred to the DXA machine (Norland XR46 Densitometer Machine). The pigs were scanned in ventral-recumbency, with hind legs extended and forelegs positioned caudally. Whole body mode was used to scan and the scan was subsequently analysed using whole body analysis. Measurements made by DXA included total tissue mass, lean tissue mass, fat tissue mass and bone mineral content. After scanning the pigs

were placed in a recovery room until they were able to stand and were then returned to their pens. The pigs were given their respective diets on return to their individual pens.

Blood analysis

Blood samples (2x10 mL in lithium heparin tubes) were collected from all pigs on Day 0, 3, 7, 10, 14, 21 and 28. The blood samples were centrifuged at 2,000 g for 15 minutes to recover plasma and were stored at -20°C until analysed. Plasma urea nitrogen was quantified using a commercial kit (Olympus Kit Cat. No. OSR6134). The assay was performed on an automated analyser according to the manufacturer's instructions (Olympus AU400; Olympus UK Ltd, Hertfordshire, United Kingdom). Plasma testosterone was measured using an extraction tritiated radioimmunoassay. Coefficients of variation within and between assays were 5.0% (2.58 ng/mL), 4.3% (1.08 ng/mL) and 4.6% (0.38 ng/mL). The lower limit of detection was 0.02 ng/mL.

Objective Pork Quality

pH decline in the *Longissimus* muscle was measured at 45 mins, 2 hr and 4 hr post-exsanguination using a portable pH/temperature meter (Cyberscan pH 300, Eutech Instruments,Singapore) fitted with a polypropylene spear-type gel electrode (Ionode IJ44, Ionode Pty Ltd, Brisbane, QLD) and a temperature probe.

At 24 hours post-slaughter a section of the *Longissimus thoracis* muscle was removed from the left hand side of the carcass between the 12^{th} and 13^{th} rib. For determination of pH, temperature and drip loss a 2 cm steak was cut from the appropriate sample. The muscle pH was measured as described above. Drip loss was measured using a modification of the method described by Rasmussen and Andersson (1996). The muscle was cut to a 50 g cube then wrapped in netting and suspended in a sealed plastic container. The samples were stored for 24 h at 4°C. The sample was then removed and gently patted dry to remove excess moisture before being re-weighed. Colour (L*, a* and b*) was measured with a Minolta Chromameter CR-400 (Minolta, Osaka, Japan), using D65 illumination, a 2° standard observer, and an 8-mm aperture in the measuring head, standardised to a white tile after a bloom time of 10 min. An 80±10 g sample was cut from the loin samples to measure cooking loss and shear force (Bouton, Harris, & Shorthose, 1971). The samples were then frozen in individual bags. The bagged frozen samples were then suspended from a metal rack and placed in a water bath which had been pre-heated to 70°C. The samples were then cooked at 70°C until an internal temperature of 70°C was reached (approximately 30 minutes). After removal from the water bath, the samples were allowed to cool in iced water for 30 min, patted dry to remove excess moisture, and re-weighed before being refrigerated at 4°C overnight. Cooking loss percentage for each sample was determined by dividing the difference in the raw and cooked weights by the weight of the raw pork sample. The cooked sample was then cut into five cross-section samples (1 cm^2) parallel to the muscle fibres. Warner Bratzler shear force was measured using a Warner Bratzler shear blade fitted to a Lloyd Texture Analyser (TA-2, United Kingdom). A 50 g sample of LT muscle, trimmed of visible fat and skin, was used to determine the percentage of intramuscular fat via the Ankom method (extraction of crude fat using petroleum ether) (Silliker Australia, Sydney, Australia).

Sensory Pork Quality

Eight pigs were used for each of the eight treatment combinations, a total of 64 pigs in the study. At the time of sensory analysis, samples from four pigs were

removed due to unforeseen circumstances (only six pigs used for treatment 8 and seven pigs used for treatments 3 and 5), giving a total of 60 pigs. One primal cut (loin) and one cooking method (grilling as steaks) were evaluated. One loin primal was obtained from each pig which was cut into four loin steaks of 25 mm thickness; a total of 240 loin steaks. Panellists evaluated four loin steaks in each sensory session, requiring 60 consumers.

Sensory Evaluation

The consumer panel was designed to determine sensory attributes of aroma, tenderness, juiciness, flavour and overall liking for each pork loin steak sample assessed. Demographic information (gender, household size, age, current purchasing, cooking and consumption habits of fresh pork) was captured for each consumer along with a quality grade and re-purchase intention score for each pork sample evaluated. The consumer sensory sessions were conducted at one central location, being the University of South Australia (UniSA) city east campus, Adelaide.

Consumer Recruitment

Consumers were recruited by an independent recruitment company (Intuito Market Research). The process for recruitment included emailing their extensive database of consumers, as well as running advertisements in local Adelaide newspapers (The Advertiser and Sunday Mail), social media (Facebook and Intuito webpage) and contacting specific community and business groups directly.

The specifications for recruitment were that all participants needed to be consumers who had eaten fresh pork (not bacon or ham) in the past month and who were aged between 18 and 65 years. Butchers and people working in meat production and sales were excluded. Individuals (n=60) were asked to join a panel for approximately 30 minutes, at session times of 10.00 am, 12 pm, 1.30 pm and 3 pm on two pre-determined days in February 2014.

Participants registered with Intuito either online or over the telephone and were contacted by a recruiter to arrange a time that would suit them to attend. Participants were given an honorarium for their participation in the study and were used only once.

Allocation of Frozen Samples into Sensory Sessions

Frozen pork loin steak samples were received at the SARDI Waite campus on 8 January 2014, in individual, vacuum sealed packages. Samples were sorted into their respective sensory sessions and placed in a freezer at -18°C. Copies of a one page session labelling document were prepared in advance for every session to indicate which loin steaks were required for the sessions. The document listed the session number and 4-digit code assigned to each loin steak along with treatment details.

Thawing and Preparation Protocols

The carton required for each day of sensory sessions was removed from the -18°C freezer and placed into the 4°C constant temperature room for 48 hours. Samples were prepared for the sensory sessions between one and five hours after removal from the 4°C room. On removal from the carton, the 32 individual loin steak samples required for the session (16 for the final session) were checked against the session labelling document to ensure the session contained the correct samples. The 4-digit number was used as the primary identification tool. Each sample was identified by its unique 4-digit number and this ID followed the sample

after removal from its vacuum packaging to presentation to the sensory panellist for evaluation.

Grilling Protocol

The loin steaks were removed from the vacuum packaging, labelled with their 4-digit number, placed onto a tray and stored at 5°C until required. The temperature of the loin steaks was 5-7°C before cooking commenced. The four samples to be evaluated by each consumer were in a randomised tasting order so the cooking of samples could not be done to order. The loin steaks were grilled in groups of four loin steaks with eight grilling batches required for each session.

The grill used for this study was a Silex Grill Model GTTPowersave 10.10-30 (Silex Elektrogerate GmbH, 22143 Hamburg, Germany). The grilling protocol was that previously developed and utilised in Pork CRC project 3A-103 to produce grilled steaks cooked to an end point temperature of 70°C after a two minute resting period. The grilling started approximately 40 minutes before the start of each session. For every group of loin steaks grilled, the internal take-off and resting temperature was measured for one steak cooked to ensure the equipment was functioning as expected and the required end point temperature of 70°C was achieved. The grilling and resting times were measured with digital timers.

Once the steaks had been grilled for the required amount of time, they were removed from the grill and placed next to their ID label on the cutting board for resting. This process was repeated until all steaks had been cooked for the session. In between each sensory session the grill was switched off and the plates thoroughly cleaned with hot water and detergent.

Presentation Protocol

The preparation room was maintained at a temperature of 23°C during the sensory sessions. In each session, the samples (n=4) evaluated by each consumer were in a randomised tasting order so all samples needed to be prepared and ready to serve for the start of the sample evaluation section of the sensory session; approximately 10 minutes after the start of the session. The samples could not be prepared to order. Some samples were stored in the containers for up to 30 minutes prior to consumer evaluation. To keep the prepared samples warm during the evaluation and prevent moisture loss, they were stored in sealed and labelled glass Pyrex containers (World Kitchen, Rosemont, Illinois, USA) on top of heated warming plates from Cuisinart (Model CWT-240A, NSW, Australia). At the start of the day, the warming plates were preheated to the 65°C setting and the Pyrex containers placed on top. After two minutes resting on the cutting board, four steaks (grilled at the same time) were trimmed on all four sides to remove the fat and edges and the centre pieces used for consumer evaluation. These were transferred with their labels to sealed Pyrex holding containers. This process was repeated for the 32 steaks required for each sensory session (16 for the final session).

Serving of Samples to Consumers

A one page serving order document was prepared for each of the 60 consumers in the study. This document contained the order in which the four samples were to be tasted (identified by order, sample description and 4-digit identification number) by each panellist in each session. Before the start of each session, the page corresponding to the correct session and panellist was secured above the booth in the preparation room. The four sample plates were also pre-labelled with the 4-digit sample numbers and stacked in the correct tasting order next to the

booth. The consumers were instructed to switch on a light in their booth once they were ready to evaluate a sample of pork. This action illuminated a duplicate light in the preparation room which served as a signal to commence the serving process to that consumer. Two people undertook the serving of samples in a sensory session.

When a consumer was ready for a sample and the light was illuminated, the server would identify the panellist and sample number required. They would:

1. collect pre-labelled sample plate from beside the tasting booth.
2. locate the correct sample in the Pyrex container.
3. undertake a number identification check between plate and Pyrex container.
4. place sample onto plate.
5. open the serving hatch and present the sample to the consumer.
6. switch off the light beside the tasting booth.
7. cross out the sample ID number on the serving order document.

This process was repeated for all four samples and the protocol followed for all sensory sessions. The serving operation was completed in approximately 10 to 20 minutes per session.

Consumer Evaluation

For seven of the eight sensory sessions, eight consumers evaluated four pork samples (32 tastings). The final session contained four consumers who evaluated four pork samples. The consumers were given a short briefing on the sensory evaluation process and then taken to the sensory evaluation room and placed in the individual tasting booths to start the session. Panellists recorded assessments by touch screen through the use of a computerised sensory evaluation program, Compusense Five version 5.2 (Guelph, Ontario, Canada). The session commenced with consumers answering a number of questions designed to capture individual demographic information which included: gender, household size, age, current purchasing, cooking and consumption habits of fresh pork. Consumers were then presented with each pork sample for evaluation on a numbered plastic plate. They were first asked to enter the 4-digit identification number for the sample, observe the aroma and rate this on a line scale. They were then asked to eat most of the sample before scoring for tenderness, juiciness, flavour and overall liking.

Consumers assessed the eating quality attributes of the pork samples using a continuous line scale as per Australian Standard for Sensory Analysis (AS 2542, 2007). This method provided panellists with an opportunity to express small differences in judgment as they marked the line in the position corresponding to perceived intensity for that attribute. Although potentially a more difficult task for the consumer than using a category scale, the line scale permits unlimited fineness of differentiation among consumer assessments. To ensure that the scale was easily understood by panellists, word anchors were carefully selected for each attribute and prior to the evaluation of pork samples, consumers completed a number of line scale practice assessments.

The continuous line scales used for the five quality attributes were anchored at each end with words, with the left hand side equivalent to 0 and right hand side equivalent to 100. Numerical intensity values were not shown to the consumers:

1) Aroma liking: Dislike extremely to Like extremely.
2) Tenderness: Not tender to Very tender

3) Juiciness: Not juicy to Very juicy
4) Flavour liking: Dislike extremely to Like extremely
5) Overall liking: Dislike extremely to Like extremely

Each consumer graded the samples for quality using one of the following categories:

1) Unsatisfactory (this was terrible, I did not enjoy it all)
2) Below average (this was not nice, I did not enjoy it)
3) Average (this pork was nice, I somewhat enjoyed it)
4) Above average (this pork was really nice, I enjoyed it)
5) Excellent (this pork was excellent, I really enjoyed it)

Each sample was rated for repurchase intention using one of the following categories:

1) I definitely would not buy it
2) I would probably not buy it
3) I might buy it
4) I would probably buy it
5) I would definitely buy it

Consumer assessment progress was monitored remotely on a computer running the Compusense software and assistance was provided when required. Consumers could not move onto the next question or assessment until the previous answer or assessment was completed. The session was completed once all consumers had assessed and rated their four samples.

Statistical analysis

General analysis of variance was performed with the GENSTAT 15 program (VSN International Ltd, Hemel Hempstead, UK) to analyse the main effects of sex, initial weight and feeding regime on growth performance and carcass and objective meat quality. Batch was used as a block in the analysis. For testosterone and plasma urea nitrogen a square root transformation was performed as the residual plots indicated variation was increasing with larger fitter values. A level of probability of less than 0.05 was used to determine statistical difference between the means. Fisher's-protected least significant differences were used to determine differences between treatments. Data from non-significant interaction terms is not presented.

Analysis of variance was also performed using R (R version 2.14.0, http://www.r-project.org) to analyse the main effects of sex, weight and feeding regime on sensory meat quality. Regression analysis was used to predict overall liking of pork based on the four attributes of tenderness, aroma, juiciness and flavour and was also used to determine whether quality grade score and re-purchase intention could be predicted from sensory variables assessed in this study. Sex differences for fail rate for quality grade and re-purchase intention were examined using chi-square analysis (Microsoft Excel 2007).

3. Outcomes

This experiment required the relocation of a Dual Energy X-ray Absorptiometry (DXA) machine to Medina Research Station and the establishment of a new standard operating procedure to undertake the DXA procedure. The major issue was identifying the most appropriate drugs to use to sedate the animals within 24 hours of slaughter for human consumption. This required a separate experiment that was conducted under veterinary and animal welfare supervision, but this was

very successful and has now been used by others involved in pig research at DAFWA. It confirmed that Stresnil® at 2 mL/10 kg can be used to successfully sedate pigs from 20 to 120 kg for the DXA scan, with pigs suitable for human consumption within 24 hours.

Growth performance and carcass quality

The effect of sex, feeding regime and starting weight on growth performance and carcass quality are shown in Table 3. Sex did not affect LW on Day 0, 14 or 28 ($P>0.05$; Table 3). Daily gain, feed intake, feed conversion ratio, carcass weight (CW) and dressing percentage (DP) were also unaffected by sex at any time period ($P>0.05$).

Pigs fed the restricted diet had a lower daily gain ($P<0.001$), feed intake ($P<0.001$), CW ($P<0.001$), DP% ($P=0.023$) and P2 ($P<0.001$) compared to pigs fed *ad libitum* for all time periods. Feed:gain was not affected by feeding regime at any time period ($P>0.05$).

As expected the light pigs had a lower LW at Day 0 ($P<0.001$), 14 ($P<0.001$) and 28 ($P<0.001$) compared to the heavy pigs. They also had a lower daily gain and feed intake for all time periods ($P<0.001$). Commencing LW had no effect on feed:gain for Day 0 to 14 and Day 14 to 28 ($P>0.05$). However, light pigs had a better feed:gain from Day 0 to 28 than heavy pigs ($P=0.044$). Carcase weight ($P<0.001$), DP ($P=<0.001$) and P2 ($P=0.001$) were all lower in the light versus heavy weight pigs.

Immunised males on a restricted diet had a lower final LW and CW than entire males on a restricted diet ($P=0.018$ and $P=0.011$, respectively; Figure 6). There were no differences in final LW between immunised males and entire males when fed an *ad libitum* diet. From Day 14 to 28 and from Day 0 to 28 there was an interaction in that when their feed intake was restricted immunised males had a lower daily gain (-122 g/day and -100 g/day, respectively) compared to entire males ($P=0.011$ and $P=0.011$, respectively; Figures 1 and 2). There was no difference in daily gain between immunised males and entire males when the feed intake was *ad libitum*. Immunised males fed *ad libitum* were also 2 mm fatter at the P2 site than entire males ($P=0.028$, Figure 7). There was no difference in backfat between immunised males and entire males when fed the restricted diet.

When fed *ad libitum* immunised males ate 244 g/day more feed than entire males from Day 14 to 28 ($P=0.048$, Figure 3). There was no difference in feed intake between immunised males and entire males when fed a restricted diet. When pigs were fed *ad libitum* there was no difference in daily gain for heavy or light pigs from Day 14 to 28. However, light pigs grew 233 g/day slower than heavy pigs when fed a restricted diet ($P=0.039$, Figure 4). There was also a larger difference in feed intake between *ad libitum* and restricted diets in the light pigs compared to the heavy pigs ($P=0.038$, Figure 5).

There was no difference in ultrasound backfat for sex and feeding regime for any time period ($P>0.05$, Table 4). Light pigs had less backfat on Day 0 ($P<0.001$), 14 ($P<0.001$) and 28 ($P<0.001$) than heavy pigs.

Table 3 - The effect of sex, feeding regime and initial starting weight on liveweight, daily gain, feed intake, feed:gain, carcass weight, dressing percentage and P2 backfat (n=8).

	Sex		Feeding regime		Weight		SEM[a]	P-value						
	E	I	Adlib	Restrict	Light	Heavy		S	F	W	S*F	S*W	F*W	S*F*W
LW (kg)														
Day 0	62.5	61.7	62.1	62.0	45.9	78.3	1.04	0.294	0.862	<0.001	0.167	0.186	0.651	0.221
Day 14	74.8	73.3	76.3	71.9	56.4	91.7	1.32	0.114	<0.001	<0.001	0.136	0.607	0.855	0.207
Day 28	87.9	86.3	91.4	82.8	68.4	105.8	1.71	0.176	<0.001	<0.001	0.018	0.199	0.382	0.428
Daily gain (kg/day)														
Day 0-14	0.88	0.83	1.01	0.70	0.75	0.96	0.050	0.146	<0.001	<0.001	0.434	0.313	0.742	0.561
Day 14-28	0.94	0.93	1.09	0.78	0.86	1.00	0.059	0.787	<0.001	<0.001	0.011	0.069	0.039	0.699
Day 0-28	0.91	0.88	1.05	0.74	0.80	0.98	0.052	0.231	<0.001	<0.001	0.011	0.422	0.060	0.935
Feed intake (kg/day)														
Day 0-14	2.33	2.27	2.73	1.87	1.93	2.67	0.116	0.416	<0.001	<0.001	0.856	0.918	0.661	0.844
Day 14-28	2.56	2.64	3.05	2.15	2.30	2.89	0.114	0.326	<0.001	<0.001	0.048	0.707	0.038	0.957
Day 0-28	2.44	2.45	2.89	2.01	2.11	2.78	0.095	0.923	<0.001	<0.001	0.191	0.871	0.319	0.880
Feed:gain														
Day 0-14	2.64	2.83	2.72	2.76	2.67	2.80	0.217	0.221	0.781	0.415	0.287	0.359	0.397	0.670
Day 14-28	2.78	2.91	2.85	2.84	2.75	2.94	0.166	0.284	0.974	0.113	0.094	0.055	0.712	0.893
Day 0-28	2.68	2.82	2.76	2.74	2.67	2.83	0.115	0.095	0.875	0.044	0.069	0.779	0.379	0.728
CW (kg)	58.4	57.1	61.0	54.5	44.6	71.0	1.23	0.142	<0.001	<0.001	0.011	0.223	0.921	0.618
DP (%)	66.3	66.0	66.5	65.7	65.2	67.1	0.769	0.620	0.159	0.001	0.442	0.941	0.403	0.543
P2 (mm)	9.06	9.87	10.3	8.59	7.81	11.1	0.700	0.109	<0.001	<0.001	0.028	0.256	0.991	0.608

[a] Standard error of the mean for Sex × Feeding Regime × Weight

Table 4 - The effect of sex, feeding regime and initial starting weight on ultrasound backfat depth (mm) for Days 0, 14 and 28 and the change for the periods 0 to 14, 14 to 28 and 0 to 28 (n=8).

	Sex		Feeding regime		Weight		SEM[a]	P-value						
	E	I	Adlib	Restrict	Light	Heavy		S	F	W	S*F	S*W	F*W	S*F*W
Day 0	8.44	8.22	8.29	8.38	7.34	9.32	0.408	0.441	0.760	<0.001	0.928	0.760	0.602	0.462
Day 14	9.84	9.53	9.97	9.41	8.31	11.06	0.621	0.479	0.205	<0.001	0.396	0.671	0.671	0.777
Day 28	10.7	10.8	11.1	10.4	8.97	12.6	0.648	0.734	0.123	<0.001	0.123	0.635	0.311	0.542
Δ Day 0-14[b]	1.27	1.31	1.55	1.03	0.97	1.62	0.541	0.918	0.177	0.096	0.217	0.701	0.668	0.918
Δ Day 14-28[b]	0.84	1.31	1.16	1.00	0.66	1.50	0.570	0.250	0.700	0.041	0.398	0.318	0.488	0.700
Δ Day 0-28[b]	2.22	2.62	2.81	2.03	1.62	3.22	0.603	0.343	0.073	<0.001	0.097	0.713	0.422	0.824

[a] Standard error of the mean for Sex × Feeding Regime × Weight
[b] Δ = change in P2 backfat

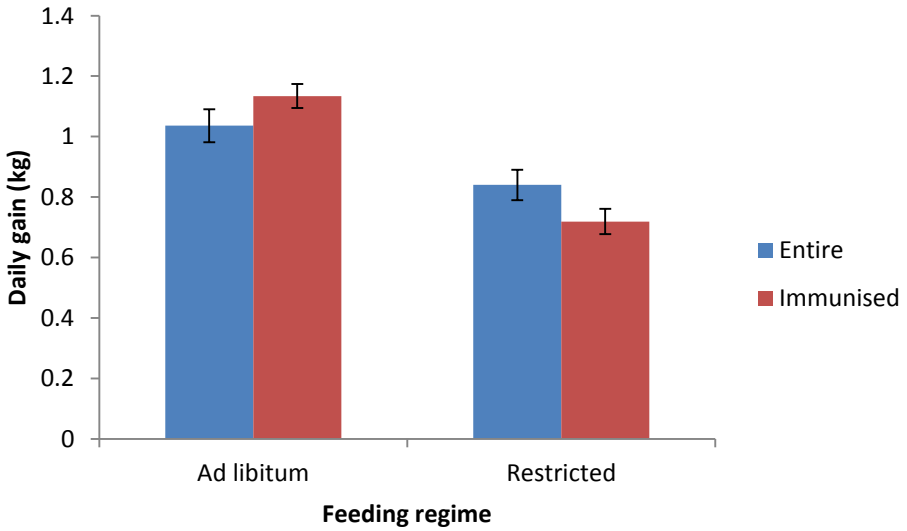

Figure 1 - The interaction between sex and feeding regime on daily gain from Day 14 to 28 (P=0.011).

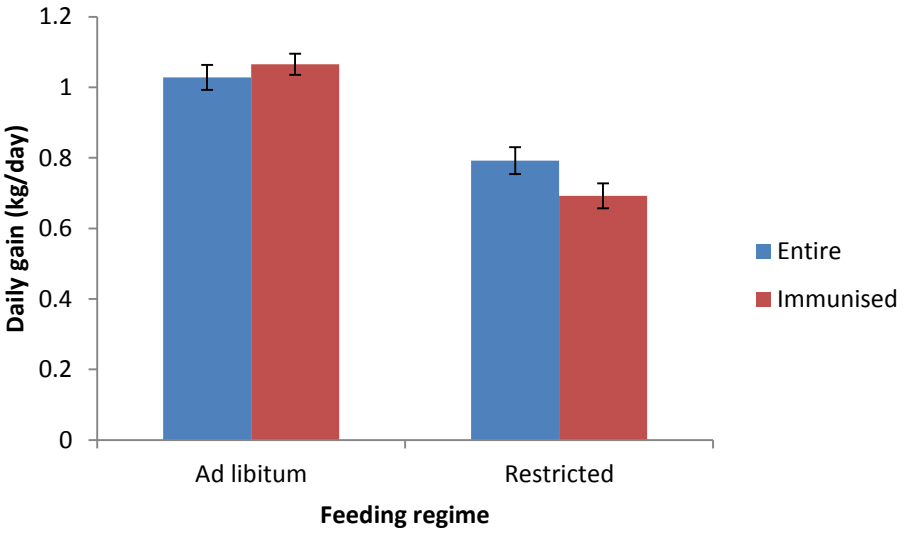

Figure 2 - The interaction between sex and feeding regime on daily gain from Day 0 to 28 (P=0.011).

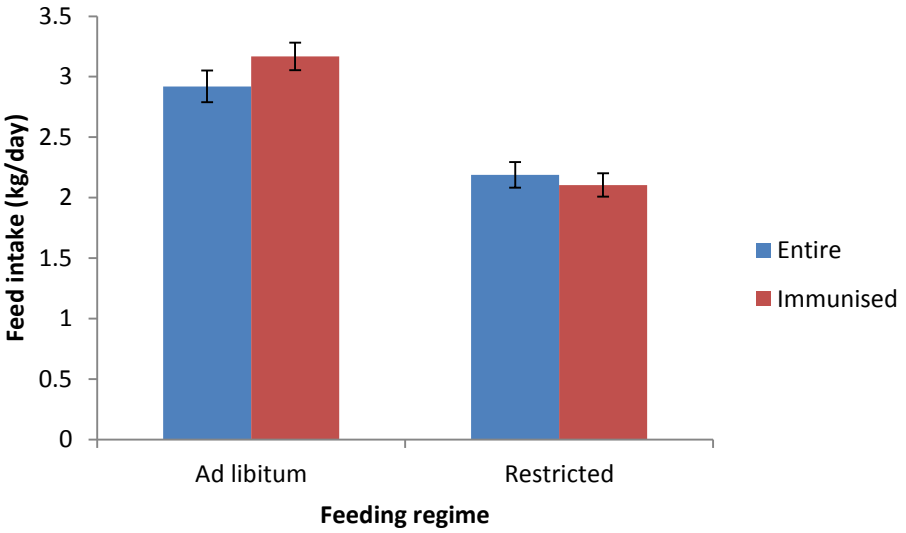

Figure 3 - The interaction between feeding regime and sex on feed intake from Day 14 to 28 (P=0.048).

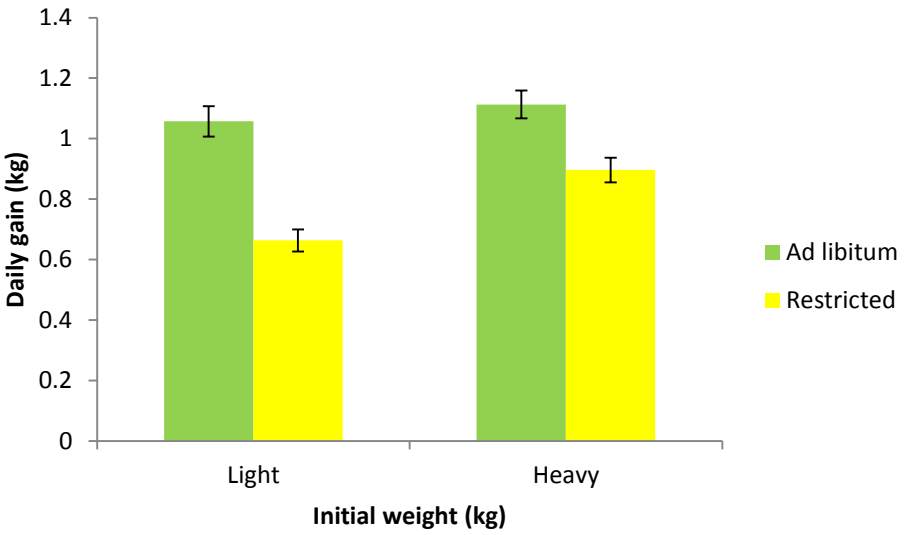

Figure 4 - The interaction between initial weight and feeding regime on daily gain from Days 14 to 28 (P=0.039).

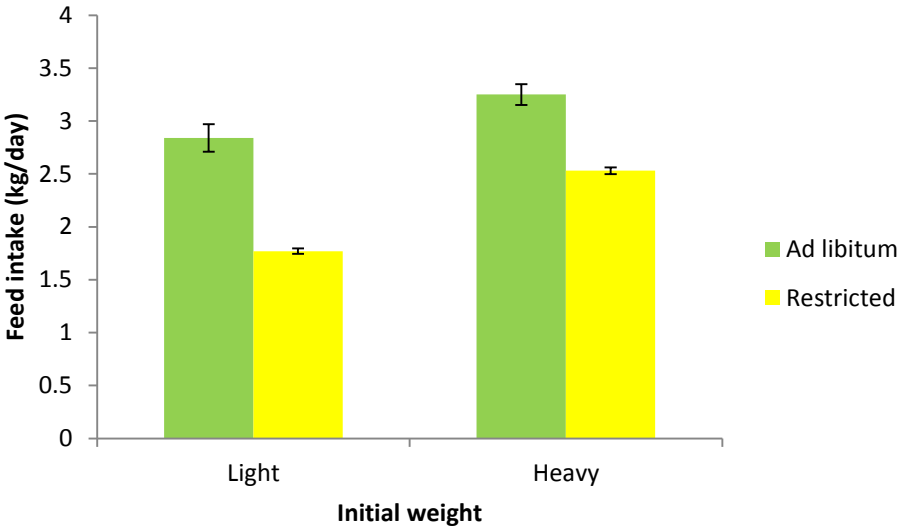

Figure 5 - The interaction between initial weight and feeding regime on feed intake from Day 14 to Day 28 (P=0.038).

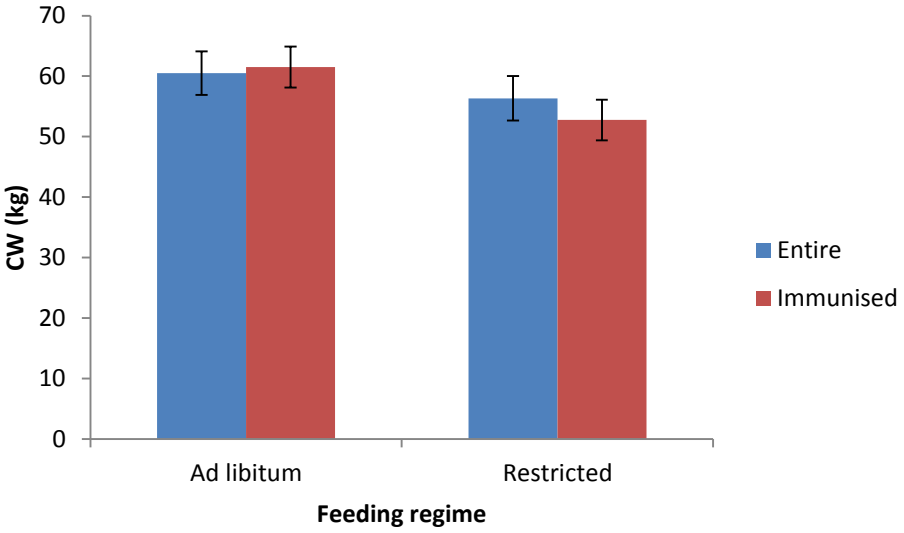

Figure 6 - The interaction between sex and feeding regime on CW (P=0.011).

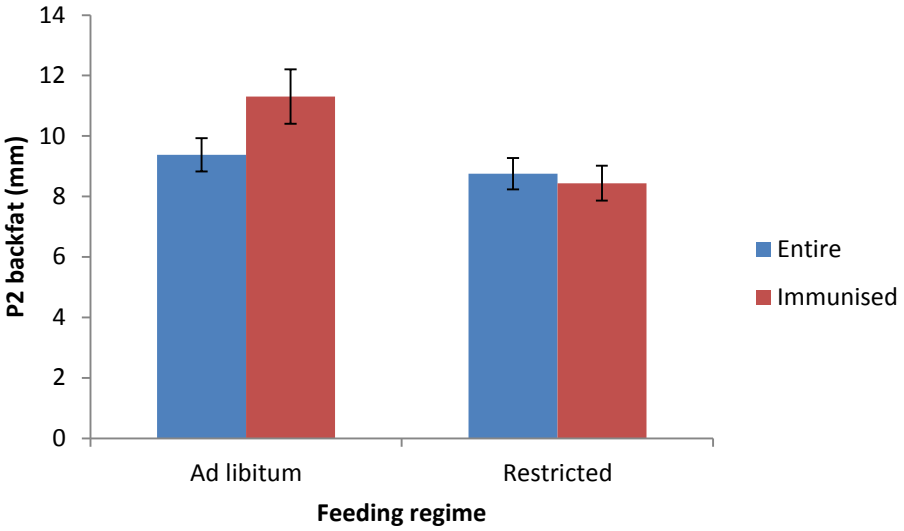

Figure 7 - The interaction between sex and feeding regime on carcass P2 (P=0.028).

Body composition

The effect of sex, feeding regime and starting weight on tissue deposition are shown in Table 5. Lean deposition was not affected by sex from Day 0 to 14 and Day 14 to 28 but for the entire period (Day 0 to 28) there was a trend for immunised males to have a reduced lean deposition compared to entire males (P=0.232, P=0.117 and P=0.067, respectively). Fat deposition was not affected by sex from Day 0 to 14, but from Day 14 to 28 immunised males deposited nearly 50% more fat than entire males (P=0.025). There was also a trend for immunised males to have an increased fat deposition over the entire period (P=0.088). Immunised male pigs had a higher fat:lean ratio compared to entire males from Day 14 to 28 and over the entire period (P=0.038 and P=0.025, respectively). Sex had no effect on ash deposition or total tissue deposition for any time period (P>0.05).

Pigs fed *ad libitum* had an increased lean deposition, fat deposition and total tissue deposition at all time periods compared to pigs fed a restricted diet (P<0.05). Ash deposition from Day 0 to 14 was not affected by feeding strategy but from Day 14 to 28 and from Day 0 to 28 ash deposition was lower in pigs fed the restricted diet (P=0.047 and P=0.021, respectively).

As expected the light pigs had a reduced lean deposition, fat deposition, total tissue deposition and fat:lean ratio compared to the heavy pigs for nearly all time periods. There was no effect of starting LW on lean deposition from Day 14 to 28 (P=0.513). Ash deposition was not affected by starting LW (P>0.05).

The heavy immunised males deposited 135 g/day less lean tissue than entire males from Day 14 to 28 (P=0.04, Figure 8). There was no difference in lean tissue deposition and total tissue deposition between entire males and immunised males in the light pigs. From Day 14 to 28 entire males deposited 187 g/day more tissue in the heavy pigs compared to the light pigs (P=0.005, Figure 9). However, the total deposition for immunised males was the same irrespective of the commencing LW.

From Day 0 to 14, entire males on a restricted diet deposited 80.8 g/day less fat than when fed an *ad libitum* diet from Day 0 to 14 (P=0.018, Figure 10). There was no difference in fat deposition for immunised males fed either a restricted or *ad libitum* diet. Immunised male pigs fed an *ad libitum* diet deposited 87.1 g/day more fat from Day 14 to 28 compared to entire males (P=0.036, Figure 11). There was no difference in fat deposition between immunised males and entire males when fed a restricted diet. Immunised males on a restricted diet had a higher fat:lean ratio than entire males but there was no difference when the pigs were fed *ad libitum* (P=0.005, Figure 14).

Light pigs receiving the *ad libitum* diet deposited 87.1 g/day more fat and had an increased fat:lean ratio than light pigs on the restricted diet from Day 0 to 28 (P=0.020 and P=0.004, Figure 12 and Figure 15, respectively). There was no difference in fat deposition between pigs fed either restricted or *ad libitum* for heavy pigs. Heavy pigs fed a restricted diet deposited 168 g/day more tissue than light pigs (P=0.019, Figure 13). The total deposition for those fed *ad libitum* was the same irrespective of the commencing LW.

There was an interaction between sex, feeding regime and initial weight from Day 14 to 28 for lean deposition. When the pigs were fed *ad libitum*, light weight immunised male pigs deposited more lean than heavy weight immunised male pigs. However, when the feed was restricted, light immunised male pigs deposited less lean compared to the others (P=0.027, Table 6).

Table 5 - The effect of sex, feeding regime and starting weight on tissue deposition as determined by DXA from Day 0 to 14, Day 14 to 28 and Day 0 to 28 (n=8).

	Sex		Feeding regime		Weight (kg)		SEM[a]	P-value						
	E	I	Adlib	Restrict	Light	Heavy		S	F	W	S*F	S*W	F*W	S*F*W
Lean deposition (g/d)														
Day 0-14	695	654	812	536	592	757	48.8	0.232	<0.001	<0.001	0.107	0.624	0.523	0.856
Day 14-28	819	671	895	686	778	802	51.4	0.117	<0.001	0.513	0.439	0.040	0.101	0.027
Day 0-28	756	708	854	610	684	780	36.6	0.067	<0.001	<0.001	0.119	0.244	0.430	0.156
Fat deposition (g/d)														
Day 0-14	86.2	86.9	108.4	64.7	55.6	117.5	21.2	0.960	0.006	<0.001	0.018	0.171	0.335	0.966
Day 14-28	90.2	135	154	71.0	81.4	144	27.5	0.025	<0.001	0.002	0.036	0.262	0.099	0.317
Day 0-28	92.6	112	132.3	73.2	71.6	134	11.6	0.088	<0.001	<0.001	0.549	0.913	0.020	0.601
Ash deposition (g/d)														
Day 0-14	10.9	10.6	11.6	10.0	10.6	11.0	1.84	0.819	0.233	0.768	0.253	0.142	0.626	0.909
Day 14-28	9.97	9.07	10.9	8.10	10.1	8.95	1.97	0.519	0.047	0.420	0.452	0.099	0.986	0.203
Day 0-28	10.5	9.88	11.2	9.14	10.4	9.99	1.25	0.482	0.021	0.650	0.829	0.891	0.754	0.240
Tissue deposition (g/d)														
Day 0-14	786	769	929	626	668	887	55.5	0.677	<0.001	<0.001	0.768	0.565	0.472	0.689
Day 14-28	920	919	1068	771	883	956	55.7	0.993	<0.001	0.072	0.068	0.005	0.019	0.096
Day 0-28	851	848	1004	695	770	929	36.4	0.885	<0.001	<0.001	0.173	0.086	0.087	0.693
Fat:Lean														
Day 0-14	0.118	0.143	0.137	0.124	0.093	0.168	0.036	0.334	0.601	0.005	0.005	0.257	0.137	0.933
Day 14-28	0.114	0.174	0.183	0.105	0.097	0.191	0.040	0.038	0.008	0.002	0.141	0.853	0.248	0.139
Day 0-28	0.118	0.156	0.156	0.118	0.096	0.178	0.023	0.025	0.021	<0.001	0.821	0.004	0.824	0.511

[a] Standard error of the mean for Sex × Feeding Regime × Weight

Table 6 - Three-way interaction in lean deposition from Day 14-28.

Feeding Regime	Ad libitum		Restricted		SEM[a]
Initial weight	Light	Heavy	Light	Heavy	
Sex					
Entire	849	971	689	768	51.4
Immunised	978	783	597	688	

[a] Standard error of the mean for Sex × Feeding Regime × Weight

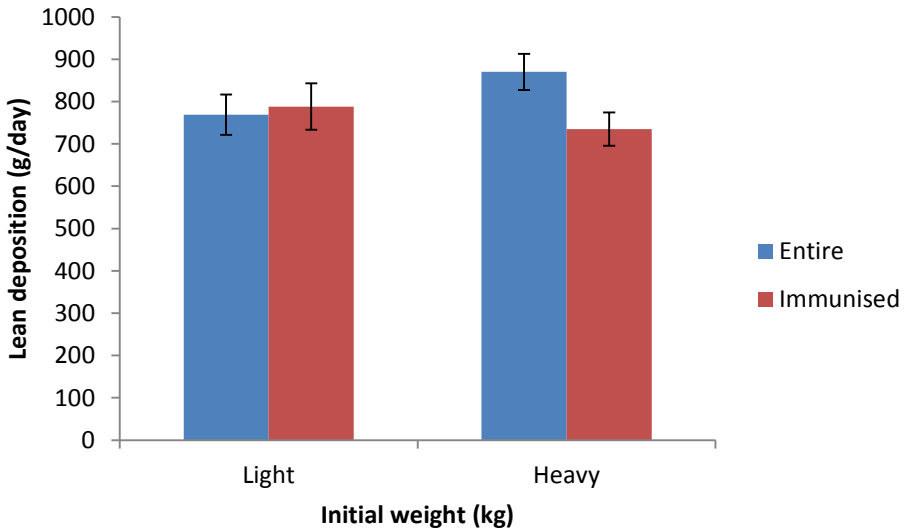

Figure 8 - The interaction between sex and initial weight for lean deposition (g/day) from Day 14 to 28 (P=0.040).

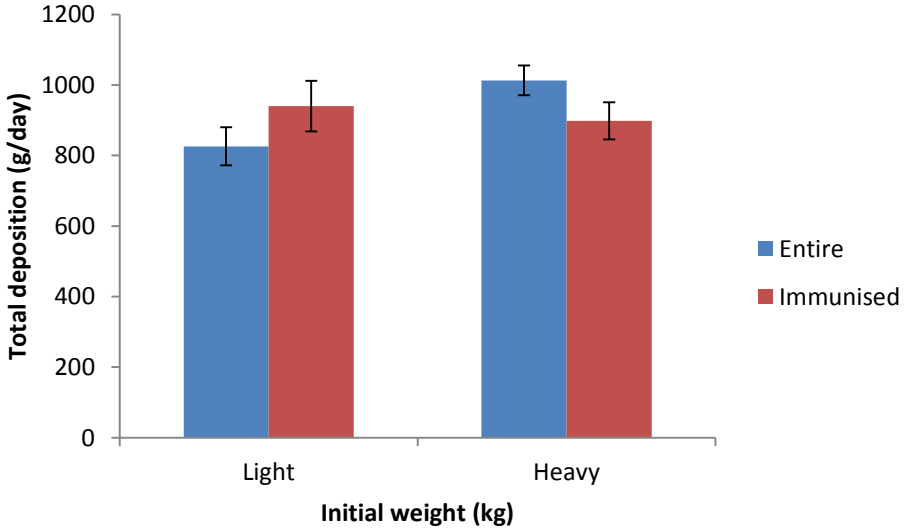

Figure 9 - The interaction between initial weight and sex on tissue deposition (g/day) from Day 14 to 28 (P=0.005).

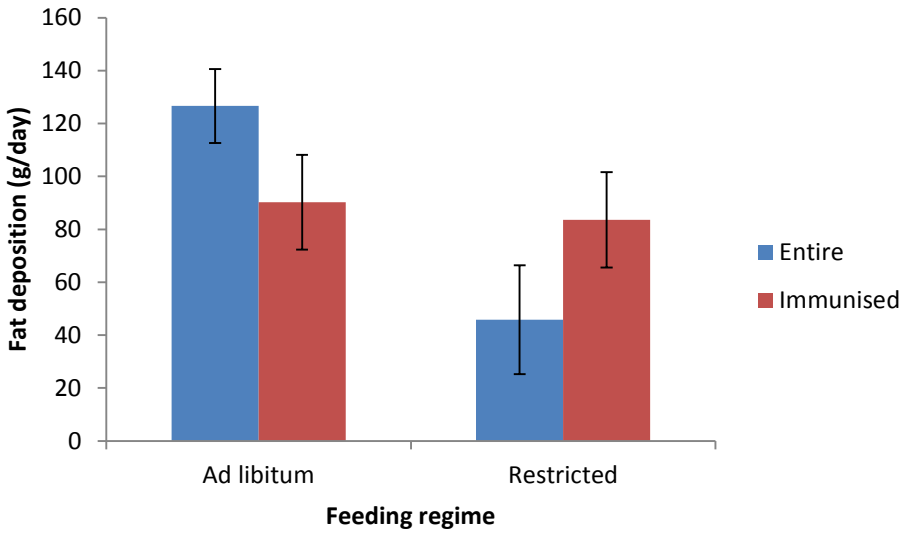

Figure 10 -The interaction between sex and feeding regime for fat deposition (g/day) from Day 0 to 14 (P=0.018).

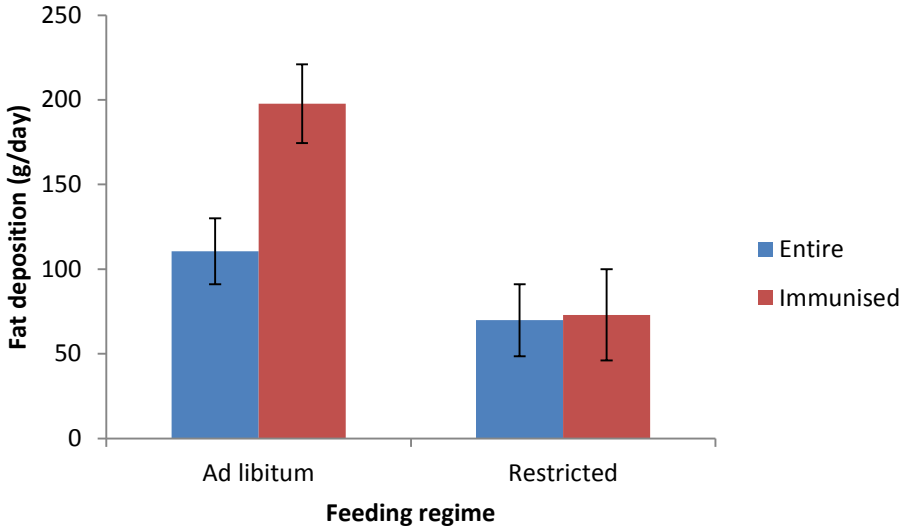

Figure 11 - The interaction between sex and feeding regime for fat deposition from Day 14 to 28 (P=0.036).

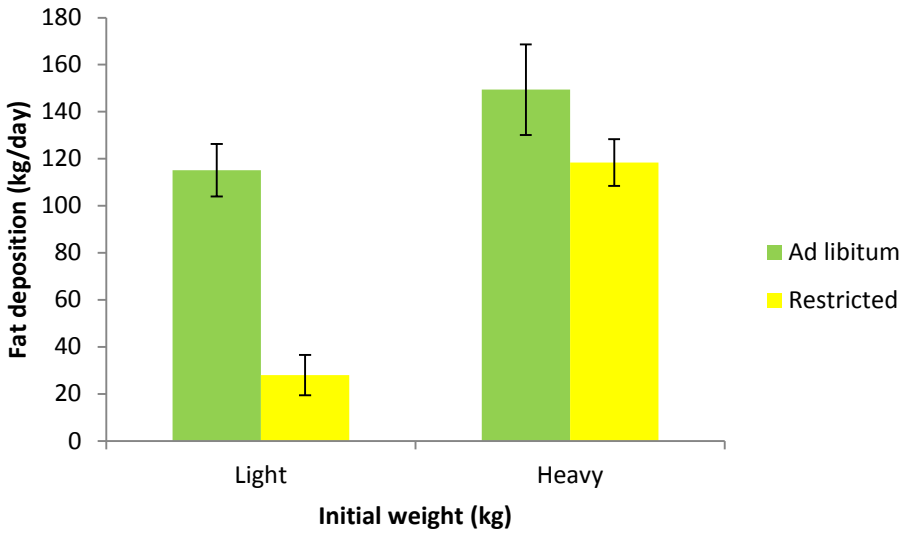

Figure 12 - The interaction between initial weight and feeding regime for fat deposition from Day 0 to 28 (P=0.020).

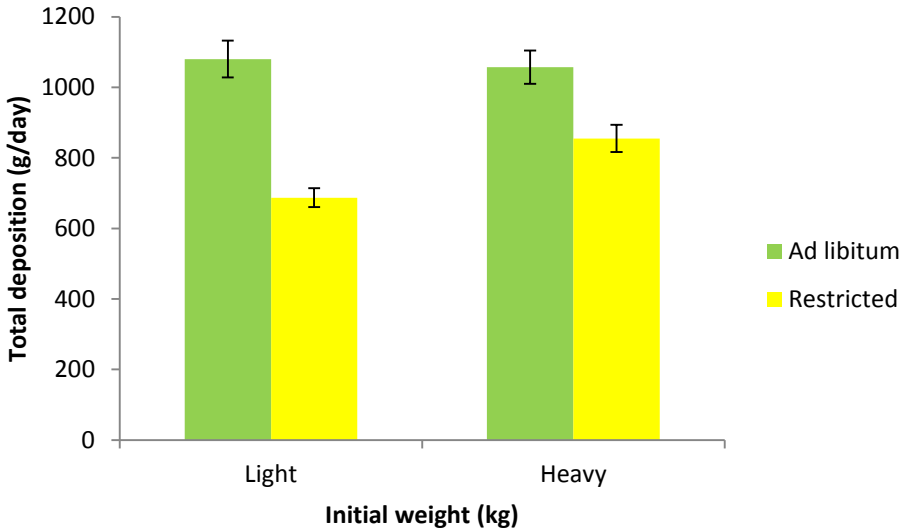

Figure 13 - The interaction between initial weight and feeding regime on total deposition (g/day) from Day 14 to 28 (P=0.019).

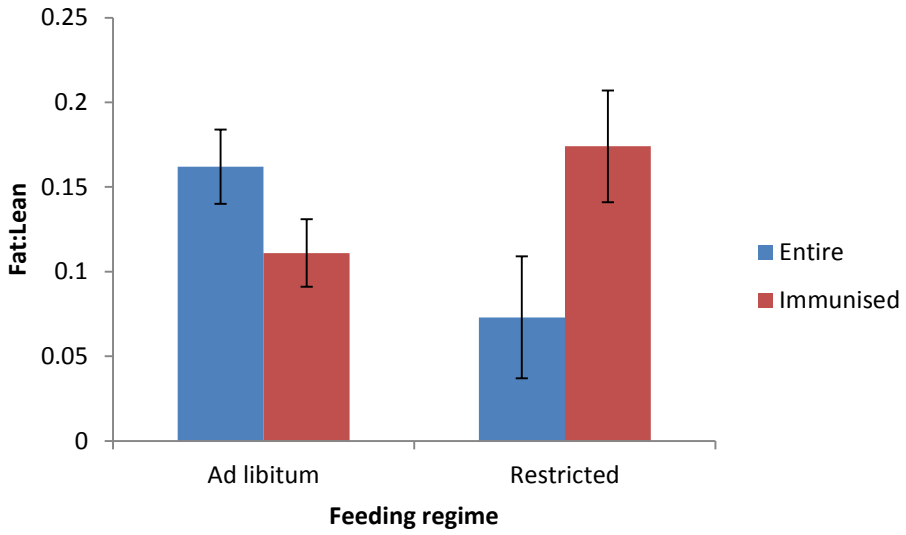

Figure 14 -The interaction between sex and feeding regime on the fat to lean ratio from Day 0 to 14 (P=0.005).

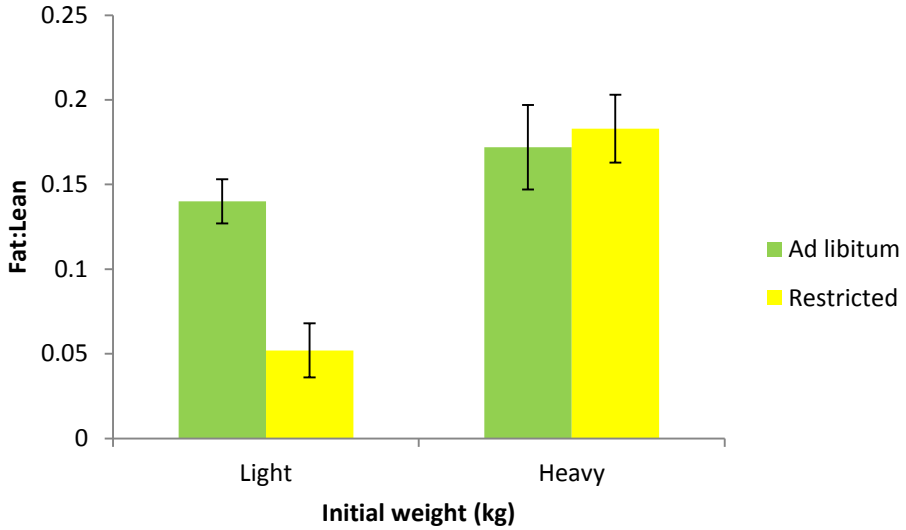

Figure 15- The interaction between initial weight and feeding regime on the fat to lean ratio from Day 0 to 28 (P=0.004).

Physiological measures

There was no difference between immunised males and entire males for testosterone levels on Day 0 and 3 (P=0.814 and P=0.153, respectively; Table 7). From Day 7 onwards immunised males had lower testosterone levels than entire males (P<0.05, Figure 16). Pigs fed *ad libitum* had increased testosterone levels on Day 3, 14 and 28 (P<0.05) compared to those on the restricted diet but there was no difference on Day 0, 7, 10 and 21 (P>0.05). On Day 3 (P=0.011) and Day 10 (P=0.042) heavy pigs had higher testosterone levels than light pigs. There was no significant difference in testosterone levels on other days. There was a feed by sex interaction on Days 14 and 28 with entire male pigs fed *ad libitum* having more testosterone than entire male pigs on the restricted diet (P=0.008 and P<0.001, respectively).

There was no difference between immunised males and entire males in PUN levels on Day 0 and 3 (P=0.761 and P=0.916, respectively; Table 8). From Day 7 onwards immunised

males had higher PUN levels than entire males ($P<0.05$, Figure 17). Pigs fed *ad libitum* had higher PUN levels than those on the restricted regime for all times except Day 0, 14 and 28 (Figure 18). On Day 14 and 28 the pigs were off feed overnight and this is the most likely explanation for the lack of difference in PUN levels between feeding regimes at these times. From Day 10 onwards PUN was higher in heavy pigs compared to light pigs (Figure 19). There was a sex by feed interaction on Day 14 with immunised males fed a restricted diet having a higher PUN than those fed an *ad libitum* diet ($P=0.036$).

Table 7 - The effect of sex, feeding regime and starting LW on testosterone (square root) over time.

	Sex		Feeding regime		Weight (kg)		SEM[a]	P-value						
	E	I	Adlib	Restrict	Light	Heavy		S	F	W	S*F	S*W	F*W	S*F*W
Day 0	1.50	1.53	1.57	1.45	1.46	1.57	0.167	0.814	0.305	0.337	0.268	0.626	0.950	0.963
Day 3	1.26	1.13	1.30	1.09	1.31	1.32	0.127	0.153	0.023	0.011	0.880	0.112	0.420	0.381
Day 7	1.13	0.50	0.84	0.80	0.78	0.86	0.120	<0.001	0.663	0.321	0.973	0.592	0.297	0.453
Day 10	1.15	0.37	0.78	0.74	0.69	0.83	0.096	<0.001	0.567	0.042	0.107	0.255	0.125	0.424
Day 14	1.26	0.33	0.92	0.68	0.76	0.84	0.111	<0.001	0.004	0.290	0.008	0.339	0.223	0.261
Day 21	1.22	0.35	0.82	0.75	0.71	0.86	0.124	<0.001	0.411	0.103	0.097	0.149	0.075	0.817
Day 28	1.44	0.36	1.05	0.75	0.88	0.92	0.130	<0.001	0.002	0.661	<0.001	0.67	0.031	0.394

[a] Standard error of the mean for Sex × Feeding Regime × Weight

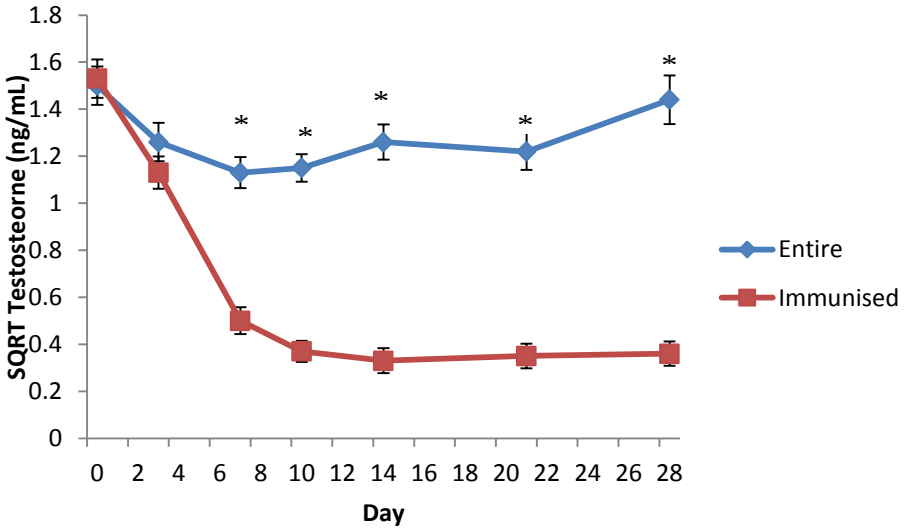

Figure 16 - The effect of sex on the change in testosterone over time.

Table 8 - The effect of sex, feeding regime and starting LW on plasma urea nitrogen (square root) over time.

	Sex		Feeding regime		Weight (kg)		SEM[a]	P-value						
	E	I	Adlib	Restrict	Light	Heavy		S	F	W	S*F	S*W	F*W	S*F*W
Day 0	1.60	1.62	1.62	1.60	1.59	1.64	0.094	0.761	0.826	0.431	0.178	0.589	0.889	0.915
Day 3	1.66	1.67	1.72	1.60	1.67	1.66	0.080	0.916	0.037	0.853	0.065	0.316	0.593	0.891
Day 7	1.69	1.78	1.84	1.65	1.72	1.77	0.076	0.044	<0.001	0.317	0.727	0.585	0.292	0.907
Day 10	1.71	1.87	1.91	1.67	1.69	1.89	0.087	0.016	<0.001	0.002	0.269	0.693	0.427	0.411
Day 14	1.61	1.84	1.70	1.75	1.61	1.84	0.080	<0.001	0.423	<0.001	0.036	0.751	0.211	0.614
Day 21	1.77	2.02	2.00	1.78	1.81	1.98	0.084	<0.001	<0.001	0.008	0.885	0.160	0.434	0.450
Day 28	1.68	1.87	1.80	1.74	1.67	1.88	0.072	<0.001	0.265	<0.001	0.862	0.419	0.157	0.820

[a] Standard error of the mean for Sex × Feeding Regime × Weight

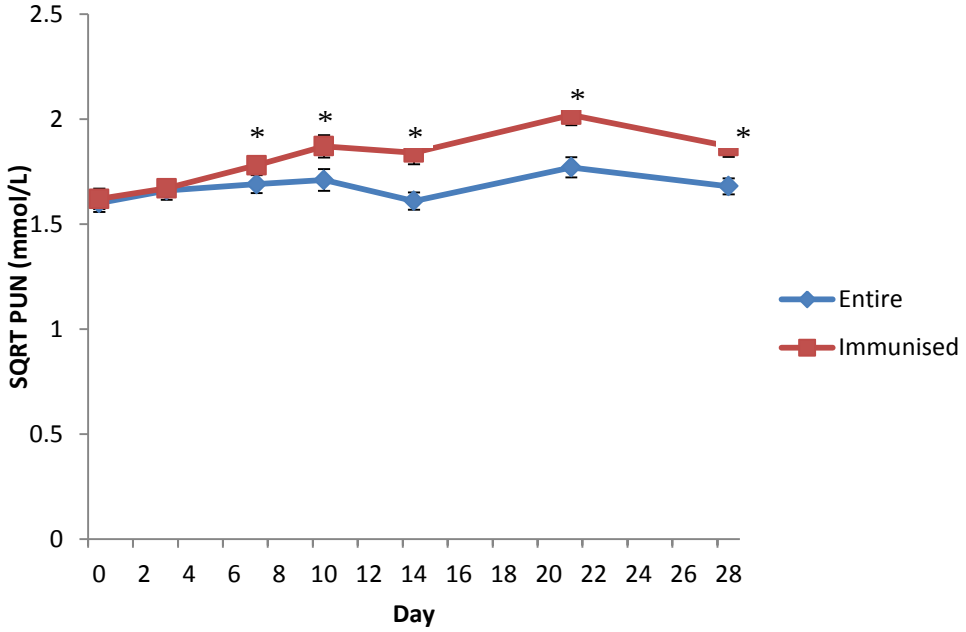

Figure 17 - The effect of sex on the change in PUN over time.

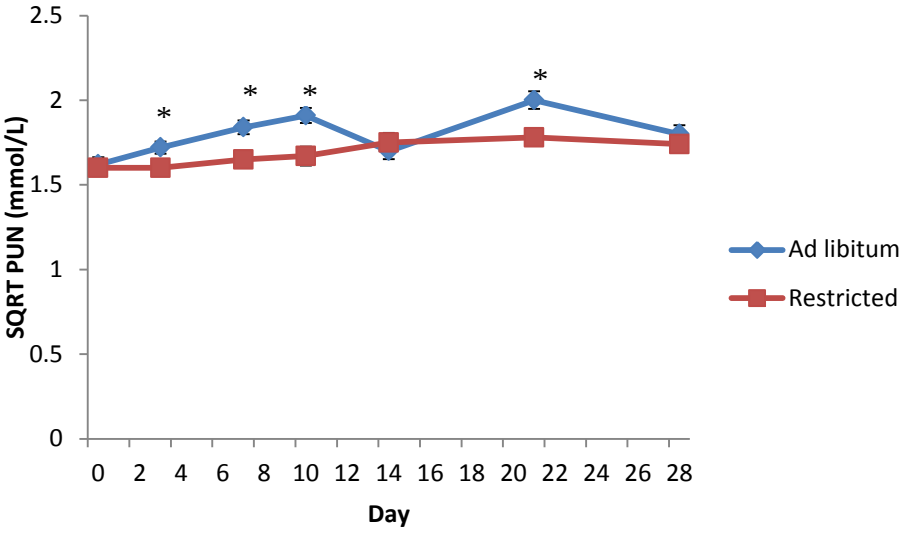

Figure 18 - The effect of feeding regime on the change in PUN over time.

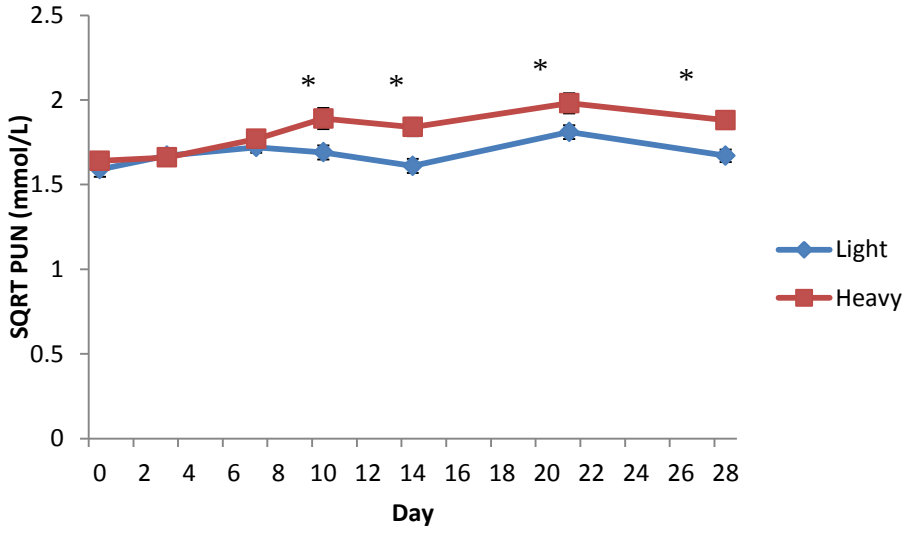

Figure 19 - The effect of starting weight on the change in PUN over time.

Objective meat quality

Table 9 shows the effect of sex, feeding regime and initial weight on pH and temperature decline from 45 mins to 24 h post-slaughter. There was no difference in the rate of pH decline for sex, feeding regime or weight at any time point post slaughter ($p>0.05$). Sex had no effect on temperature ($P>0.05$). Heavy pigs cooled more slowly than light pigs ($P<0.05$). Carcasses from pigs fed *ad libitum* cooled more slowly than those on a restricted diet ($P<0.05$).

The effect of sex, feeding regime and initial weight on objective meat quality is shown in Table 10. There was no effect of sex on objective meat quality ($P>0.05$). Pigs on the restricted diet had less intramuscular fat than those fed *ad libitum* ($P=0.008$) but there was no effect on other measures of meat quality ($P>0.05$). Light pigs had a greater drip loss ($P=0.033$) and cook loss ($P=0.003$) compared to heavy pigs. There was an interaction between sex and feeding regime in that immunised males fed *ad libitum* had 29% less drip loss than entire males fed *ad libitum* ($P=0.026$, Figure 20). There was no difference in drip loss between entire and immunised males when pigs received the restricted diet.

When fed *ad libitum* drip loss was higher with light weight entire males compared to the other treatments (P=0.044, Table 11). However, when feed was restricted drip loss was higher for light weight immunised male pigs compared to the other treatments.

Table 9 - The effect of sex, feeding regime and initial weight on pH and temperature decline from 45 mins to 24 h post-slaughter.

	Sex		Feeding regime		Weight (kg)		SEM[a]	P-value						
	E	I	Adlib	Restrict	Light	Heavy		S	F	W	S*F	S*W	F*W	S*F*W
pH decline														
45 min	6.12	6.16	6.11	6.16	6.06	6.22	0.169	0.688	0.548	0.058	0.967	0.807	0.017	0.728
2 h	5.91	5.92	5.91	5.91	5.88	5.94	0.184	0.976	0.981	0.499	0.503	0.551	0.098	0.465
4 h	5.73	5.75	5.75	5.73	5.72	5.76	0.154	0.800	0.843	0.604	0.672	0.800	0.066	0.350
24 h	5.39	5.38	5.40	5.38	5.38	5.39	0.184	0.818	0.536	0.614	0.098	0.103	0.536	0.982
Temperature (˚C)														
45 min	35.5	36.1	36.2	35.4	33.9	37.7	1.21	0.307	0.188	<0.001	0.103	0.350	0.352	0.797
2 h	22.1	22.2	23.0	21.3	19.9	24.4	0.054	0.927	0.007	<0.001	0.881	0.720	0.186	0.267
4 h	12.5	12.5	13.1	11.9	10.7	14.3	0.798	0.932	0.005	<0.001	0.274	0.470	0.423	0.230

[a] Standard error of the mean for Sex × Feeding Regime × Weight

Table 10 - The effect of sex, feeding regime and initial weight on objective meat quality measures.

	Sex		Feeding regime		Weight (kg)		SEM[a]	P-value						
	E	I	Adlib	Restrict	Light	Heavy		S	F	W	S*F	S*W	F*W	S*F*W
pH ult	5.39	5.38	5.40	5.38	5.38	5.39	0.038	0.818	0.536	0.614	0.098	0.103	0.536	0.982
Drip loss (%)	4.99	4.57	4.64	4.92	5.32	4.25	0.692	0.398	0.566	0.033	0.026	0.615	0.968	0.044
L*	50.8	51.0	50.6	51.2	51.7	50.7	1.24	0.810	0.437	0.066	0.582	0.735	0.589	0.282
a*	6.79	6.29	6.47	6.61	6.32	6.76	0.434	0.110	0.640	0.160	0.561	0.509	0.697	0.368
b*	2.45	2.24	2.33	2.36	2.25	2.44	0.377	0.436	0.893	0.484	0.330	0.793	0.453	0.294
Cook loss (%)	24.9	24.5	24.7	24.7	26.4	23.0	1.51	0.702	0.972	0.003	0.155	0.766	0.170	0.939
Shear force[b]	38.4	38.1	36.2	40.3	40.1	36.4	3.19	0.960	0.067	0.135	0.736	0.468	0.085	0.558
Intramuscular fat (%)	0.92	0.94	1.12	0.74	0.87	0.99	0.196	0.911	0.008	0.360	0.434	0.517	0.316	0.576

[a] Standard error of the mean for Sex × Feeding Regime × Weight

[b] Pre cook weight used as a covariate

Table 11 - Three-way interaction in for drip loss (%).

Feeding Regime	*Ad libitum*		Restricted		SEM[a]
Initial weight	Light	Heavy	Light	Heavy	
Sex					
Entire	6.58	4.23	4.72	4.43	0.692
Immunised	3.79	3.96	6.18	4.37	

[a] Standard error of the mean for Sex × Feeding Regime × Weight

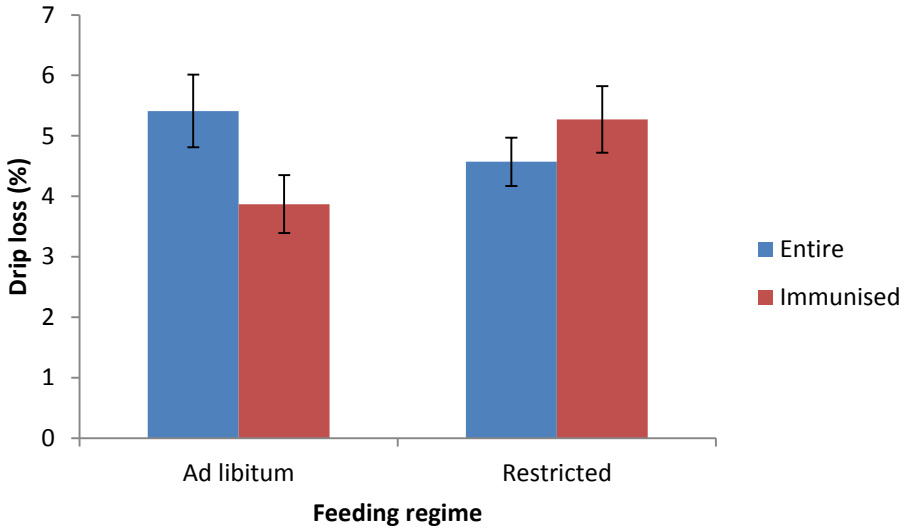

Figure 20 - The interaction between feeding regime and sex on drip loss (P=0.026).

Sensory Meat Quality

Sensory demographics

There were 60 consumers in this study of which 35% were male and 65% were female. The distribution of the ages of the consumers involved is given in Figure 21. The average household size was 3.37 people.

The consumption frequency of meals which included pork, lamb, beef, chicken and fish is given in Table 12. 73.3% of the consumers ate pork at least once per week (Figure 22). The level of appeal for pork by consumers is comparable to other meats (Table 13).

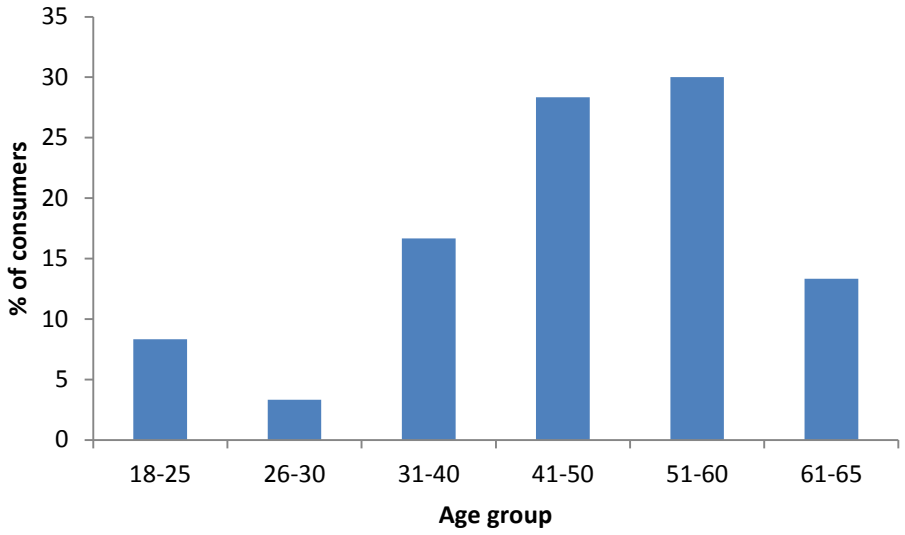

Figure 21- Percentage age distribution of consumers involved in the study.

Table 12 - Percentage consumption of meals including pork, lamb, beef, chicken or fish by the consumers in this study in the last week.

	Number of meals in the last week				
	0	**1**	**2**	**3**	**4 or more**
Pork	45	30	5	0	20
Lamb	45	15	3.3	0	36.7
Beef	43.3	25	11.7	0	20
Chicken	18.3	46.7	21.7	3.3	10
Fish	41.7	28.3	3.3	1.7	25

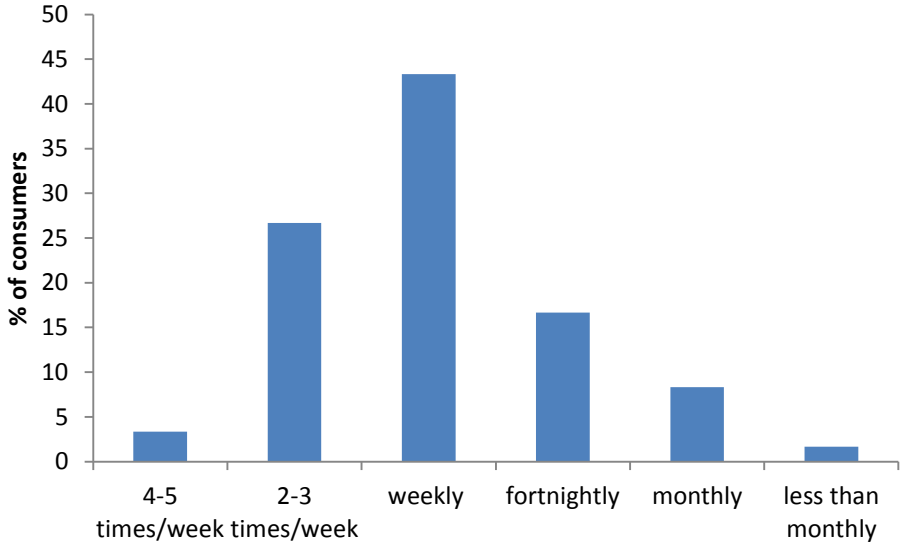

Figure 22 - The frequency with which consumers in the study ate pork.

Table 13 - The percentage level of appeal of the consumers of different meats (where 0 is extremely unappealing and 10 is extremely appealing).

	Level of appeal				
	0-4	5-6	7-8	9-10	AVG±SD
Pork	8.33	18.3	53.3	20	7.12±1.71
Lamb	15	18.3	41.7	25	6.68±2.44
Beef	6.67	20	51.7	20	7.05±1.89
Chicken	1.67	18.3	50	30	7.60±1.40
Fish	25	25	28.3	21.7	6.07±2.56

The major cuts of pork purchased by consumers involved in the sensory panels were pork loin chops/cutlets (65%), roasting cuts (48%), rashers/spare ribs (47%), sausages (42%), fillets (40%), steaks (38%), mince (33%), diced/stir fry (28%) and schnitzels (28%). The preferred method of cooking was grilled/BBQ/pan frying (88.3%), followed by roasting/baking (63%), stir frying (51.6%) and casserole/simmering (8.3%).

Consumers preferred their pork medium to well done (86.7%) with 8.3% preferring it medium rare and 5% preferring it well done (Figure 23).

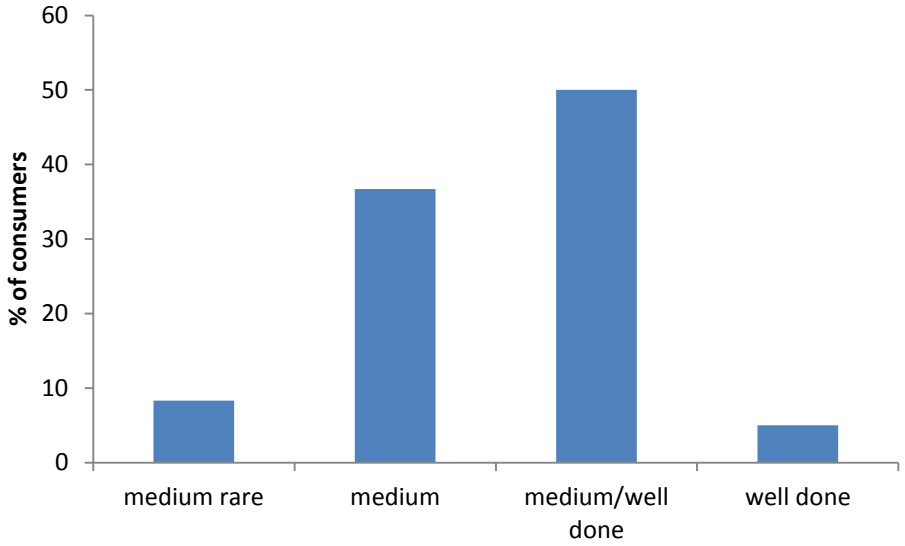

Figure 23 - The percentage of consumers preferring their pork cooked either medium rare, medium, medium/well done or well done.

Sensory results

Table 14 - Summary data for the sensory traits of aroma, tenderness, juiciness, flavor, overall liking, quality grade and repurchase intent (n=240).

	Minimum	1st quartile	Median	Mean	SD	3rd quartile	Maximum
Aroma	4.5	46.9	67.5	63.5	22.8	82.0	100.0
Tenderness	0.5	31.7	58.5	53.7	28.1	77.0	99.0
Juiciness	2.5	35.2	56.5	54.0	25.2	74.0	98.5
Flavour	2.0	39.5	57.0	55.9	23.1	73.5	98.5
Overall liking	1.5	37.4	56.0	55.1	24.8	74.6	99.0
Quality grade	1.0	2.0	3.0	3.14	0.95	4.0	5.0
Repurchase intention	1.0	2.0	3.0	3.10	1.23	4.0	5.0

The correlation coefficients between all of the sensory attributes for all evaluations are given in Table 15. Flavour and overall liking are the most highly correlated (R^2=0.896) with aroma being least correlated to all of the sensory attributes.

Table 15 - Correlation coefficients between all sensory attributes for all evaluations (n=240).

	Aroma	Tenderness	Juiciness	Flavour	Overall liking	Quality grade	Repurchase intention
Aroma	1.000						
Tenderness	0.277	1.000					
Juiciness	0.387	0.824	1.000				
Flavour	0.440	0.726	0.788	1.000			
Overall liking	0.420	0.847	0.855	0.896	1.000		
Quality grade	0.216	0.690	0.672	0.752	0.808	1.000	
Repurchase intention	0.235	0.693	0.650	0.702	0.768	0.867	1.000

Upon closer examination of the data it appears that some people give scores in a narrow range and some over a wide range. Therefore the data was re-assessed on the consumer basis (Table 16). When based on all consumers in the study the correlations between sensory attributes was higher than when based on all evaluations. Again the highest correlation was found between flavor and overall liking (R^2=0.925).

Table 16 - Correlation coefficients between sensory attributes for all consumers (n=60).

	Aroma	Tenderness	Juiciness	Flavour	Overall liking	Quality grade	Repurchase intention
Aroma	1.000						
Tenderness	0.569	1.000					
Juiciness	0.614	0.869	1.000				
Flavour	0.691	0.780	0.855	1.000			
Overall liking	0.648	0.875	0.875	0.925	1.000		
Quality grade	0.401	0.633	0.558	0.706	0.762	1.000	
Repurchase intention	0.413	0.603	0.510	0.624	0.695	0.874	1.000

Regression analysis was used to see how overall liking is related to the other sensory attributes. Overall liking was influenced by flavour, tenderness and juiciness (P<0.001, P<0.0001 and P<0.001 respectively). Aroma was not significant (P=0.092). Overall liking can be predicted using the following equation:

Overall liking = -2.79 + 0.290×tenderness + 0.168×juiciness + 0.543×flavour

Consumer response scores for quality grade and repurchase intention are given in Table 17. Quality grade score and repurchase intention agree relatively closely.

Table 17 - Quality grade scores and repurchase intention for all evaluations.

Quality grade	Repurchase intention					Total
	1	2	3	4	5	
1	8	0	0	0	0	8
2	19	32	2	0	0	53
3	0	19	57	15	1	92
4	1	0	6	44	19	70
5	0	0	0	1	16	17
Total	**28**	**51**	**65**	**60**	**36**	**240**

Linear regression was used to see if the sensory attributes can predict quality grade. Quality grade was influenced by overall liking (P<0.00005), aroma

(P=0.0002) and flavor (P=0.016). Tenderness (P=0.712) and juiciness (P=0.248) were not significant. Quality grade can be predicted using the following equation:

Quality grade score = 1.650 – 0.007×Aroma + 0.009×Flavour + 0.028×Overall liking

However, overall liking had the highest correlation and therefore the regression was rerun with just overall liking. The equation for prediction of quality grade score with overall liking was:

Quality grade score = 1.43 + 0.031×Overall liking (SD 0.56, R^2= 0.61)

Linear regression was also used to see if the sensory attributes can predict re-purchase intention. Re-purchase intention was influenced by overall liking (P<0.00005), aroma (P=0.0366) and tenderness (P=0.040). Flavour (P=0.132) and juiciness (P=0.265) were not significant. Re-purchase intention can be predicted using the following equation:

Re-purchase intention = 1.169 – 0.005×Aroma + 0.008×Tenderness + 0.040xOverall liking

As for quality grade score the highest correlation for re-purchase intention was with overall liking. Therefore the regression equation for prediction of re-purchase intention with overall liking was:

Re-purchase intention = 1.00 + 0.038×Overall liking (SD 0.50, R^2= 0.50).

The effect of sex, feeding regime and initial weight on sensory pork attributes are given in Table 18. Sex, feeding regime and initial weight did not significantly affect aroma, tenderness, juiciness, flavor, overall liking, quality grade, or repurchase intention (P>0.05). However, there is a trend for pork from immunised males to be juicier than entire males (P=0.079). Light pigs that are on the *ad lib* diet scored 1.9 points worse for aroma than light pigs on the restricted diet. For heavy pigs, those on the *ad libitum* diet scored 3.2 points worse than those on the restricted diet (P=0.042). Light pigs that were on the *ad libitum* diet scored 6.7 points better for tenderness than 50 kg pigs on the restricted diet. For heavy pigs, those on the *ad libitum* diet scored 9.8 points worse than those on the restricted diet (P=0.046). For overall liking light pigs on the *ad libitum* diet scored 6.5 points better than light pigs on the restricted diet. For heavy pigs, those on the *ad libitum* diet scored 10.8 points worse than those on the restrict diet (P=0.014). For quality grade light pigs on the *ad libitum* diet scored 0.29 points better than those on the restricted diet. For heavy pigs, those on the *ad libitum* diet scored 0.38 points worse than those on the restrict diet (P=0.023, Table 19). Immunised pigs at a lighter weight scored 0.57 points better for quality grade than entire males (P=0.044). There was no difference between immunised and entire males at the heavy weight.

The percentage scores for quality grade and re-purchase intention for entire males and immunised males are given in Table 20. Fail rates (when the score is less than 3) were 9.1% and 12% lower for immunised males for quality grade (P=0.028) and re-purchase intention (P=0.007), respectively, compared to entire males.

Table 18 - The effect of sex, feeding regime and initial weight on sensory pork quality.

	Sex		Feeding regime		Weight (kg)		SEM[a]	P-value						
	E	I	Adlib	Restrict	Light	Heavy		S	F	W	S*F	S*W	F*W	S*F*W
Aroma	63.1	63.8	63.0	64.0	63.1	63.8	3.05	0.694	0.424	0.510	0.393	0.418	0.042	0.105
Tenderness	51.4	56.2	54.0	53.5	54.1	53.4	5.38	0.187	0.775	0.379	0.846	0.640	0.046	0.264
Juiciness	51.9	56.3	53.5	54.6	53.6	54.4	3.94	0.079	0.489	0.505	0.845	0.069	0.175	0.393
Flavour	55.0	56.9	56.1	55.8	55.4	56.5	3.52	0.270	0.849	0.851	0.938	0.170	0.107	0.706
Overall liking	53.3	57.0	55.1	55.0	55.4	54.8	4.37	0.210	0.625	0.456	0.858	0.101	0.014	0.932
Quality grade	3.03	3.27	3.15	3.15	3.15	3.14	0.185	0.145	0.782	0.476	0.233	0.044	0.023	0.686
Re-purchase intention	3.00	3.22	3.18	3.03	3.12	3.08	0.245	0.207	0.967	0.533	0.462	0.197	0.095	0.462

[a] Standard error of the mean for Sex × Feeding Regime × Weight

Table 19 - Sex × weight and feed × weight interaction for quality grade.

Feeding Regime	*Ad libitum*		Restricted		SEM[a]
Initial weight	50	80	50	80	
Sex					
Entire	3.06	2.89	2.78	3.27	0.185
Immunised	3.64	2.88	3.35	3.26	

Table 20 - Percentage of consumer scores for quality grade and re-purchase intention for entire males and immunised males (n=240).

	Quality grade					Fail rate (%<3)	P-value
	1	2	3	4	5		
Entires	5.6	24.2	37.1	27.4	5.6	29.8	P=0.007
Immunised	0.8	19.8	39.7	31.0	8.6	20.7	

	Re-purchase intention					Would not purchase (%<3)	
	1	2	3	4	5		
Entires	14.5	24.2	24.2	21.0	16.1	38.7	P=0.001
Immunised	8.6	18.1	30.2	29.3	13.8	26.7	

4. Application of Research

The hypothesis that light weight pigs immunised against GnRF will have a lower fat to lean muscle ratio over time compared to pigs immunised at a heavier weight was supported. Immunisation against GnRF at heavier LW's resulted in an increase in fat deposition and a decrease in lean deposition. However, this increase in fat deposition did not occur when the second vaccination was given at 50 kg LW. In addition, the increased fat deposition associated with immunisation against GnRF at heavy LWs resulted in an increase in backfat but had no impact on IM fat. The difference in fat deposition is expected as, irrespective of sex, at heavier liveweights pigs deposit less lean and the ratio of lean to fat increases as liveweight increases (Wagner *et al.*1999). In a lifetime study of lean tissue and fat composition, Suster *et al.* (2006) found that up to 94 days of age, lean tissue deposition increased and then plateaued after this with fat deposition increasing.

The hypothesis that pigs that receive feed *ad libitum* will have an improved growth rate and increased body fat composition compared to pigs that have a restricted feed intake was supported. Pigs on the *ad libitum* diet are likely to have entered the energy dependant stage where once lean tissue deposition is maximized (as determined by the genetic potential of the pig) additional energy will be deposited as fat (Campbell, 1988). These findings are supported by McMeekan (1940) who found that animals which had been on a high plane of nutrition until 16 weeks of age and were then put on a low plane of nutrition had less bone and lean and more fat compared to those that remained on the high plane of nutrition. Ramaekers *et al.* (1996) also found that restricting diets to 18 MJ ME above the daily energy requirement for maintenance resulted in an increase in lean and a decrease in fat. Davies *et al.* (1980) found that pigs on a high plane of nutrition had more fat than low plane pigs, however, in contrast to our study they found that on the low plane diet the proportion of fat did not change for entire males but for castrates it increased. This difference may be because physical castrates were used by Davies *et al.* (1980) whereas immunised males were used in the present study.

Typically immunisation against GnRF results in an increased feed intake (Cronin *et al.* 2003; Dunshea *et al.* 2011). In this experiment immunised pigs fed *ad libitum* ate more in the second two weeks following the second vaccination but an overall increase in feed intake was not observed for the entire period. Part of the additional energy and protein as a result of the increase in feed intake in the second two weeks was likely deposited as fat as shown by the associated increase

in fat deposition in the same period (McCauley *et al*. 2003). However, one component of this experiment was restricting feed intake to try to elucidate the effect that immunisation against GnRF was having on the body composition and physiological measures compared to the effect that the increase in feed intake was having. Restricting the feed intake to 2.5 times maintenance adversely affected the daily gain of pigs immunised against GnRF to a greater extent than entire males regardless of the LW when the second vaccination of Improvac was received. This is likely because at a similar level of energy intake, entire males have faster and leaner growth as they have a higher capacity for lean tissue growth (Campbell and Taverner, 1985).

A meta-analysis of 19 data sets by Dunshea *et al*. (2013) found that immunisation against GnRF increases daily gain by an average of 119 g/day compared to entire males but this increase was not observed in this experiment. McCauley *et al*. (2003) also found no difference in growth rate in individually housed pigs immunised against GnRF.

The decrease in testosterone over time showed that the administration of the Improvac® vaccine was effective. The testosterone levels of immunised males declined within 7 days following the second dose which concurs with the findings of Bauer *et al*. (2008) and Claus *et al*. (2007). The decrease in testosterone was maintained over the entire 28 day period.

PUN levels were also elevated in immunised males one week following the second vaccination. This is supported by Bauer *et al*. (2008), Claus *et al*. (2008) and Moore and Mullan (2011). The increase in PUN concentration is because the drop in anabolic hormones results in decreased nitrogen retention (Claus *et al*. 2008). The change in PUN levels in this study over time provides further evidence that immunised male pigs should be fed as entire males for two weeks after the second vaccination and then the SID level in the diet can be decreased.

The hypothesis that pigs immunised against GnRF will have improved sensory pork quality compared to entire males at both light and heavy slaughter weights was not supported. There was no difference in any sensory quality parameters between entire males and immunised males although there was a trend for the meat from immunised males to be juicer than that from entire males. The lack of difference is in agreement with Channon *et al*. (2013) who also found no difference between entire males and immunised males for any sensory attribute. A meta-analysis by Pauly *et al*. (2012) also found no differences between entire males and immunised males for tenderness and juiciness, however they caution extending this to the whole pig populations due to the lack of homogeneity of the effect sizes across the studies in the meta-analysis. The result is in contrast with Font-i-Furnols *et al*. (2008), Font-i-Furnols *et al*. (2009) and Pauly *et al*. (2010). These studies generally found the flavor and aroma of pork from entire males to be less accepted than those from immunised males. Skatole and androstenone results are pending and these may in part help to explain the lack of differences in sensory quality.

When fail rates for entire males and immunised males were compared for quality grade and repurchase intentions, immunised males were found to have approximately 10% lower fail rates for both quality grade and repurchase intentions. Channon *et al*. (2013) also found that fail rates for quality grade and re-purchase intention were higher for entire males than immunised males, although the differences were not as large.

5. Conclusion

When pigs are immunised at a light weight (50 kg) and/or on a restricted diet they have a reduced propensity to deposit fat, however the restriction in feed intake adversely affects growth rate.

The majority of fat deposition for males immunised at heavy liveweights occurs from Day 14 to 28 after the second vaccination.

The fat deposition associated with immunisation against GnRF at heavy LWs results in an increase in backfat but has no impact on IM fat.

Gender did not influence objective or eating quality in this study. However, fail rate for quality grade (20.7% IM vs 29.8% EM) and re-purchase intention (26.7% IM vs 38.7% EM) was lower for pork from immunised males compared to entire males across all treatment combinations.

6. Limitations/Risks

This experiment was necessarily conducted with all pigs housed in individual pens, hence eliminating physical interaction between pigs. This is likely to have influenced daily feed intake which is typically higher in individually housed pigs than for pigs penned in groups. However, it is unlikely that this had a significant effect on the differences that were recorded between the different treatments.

7. Recommendations

As a result of the outcomes in this study the following recommendations have been made:

- Immunisation against GnRF can successfully improve the fail rates for quality and re-purchase intention compared to entire males, however, the problem of increased backfat and therefore carcass payment penalties remain unless pigs are sold 2 weeks after receiving the second vaccination.
- Strategies need to be developed to limit the increase in backfat and decrease in lean deposition of immunised males at heavier liveweights. Potential strategies include feeding the appropriate lysine level, using pST for 2 weeks only and feeding a low energy diet.

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