Australian Government
Department of Agriculture and Water Resources

AUSTRALIAN Pork™

Management strategies to reduce feed intake and back fat in pigs receiving Improvac®

Final Report
APL Project 2014/445

June 2016

Department of Agriculture and Food WA
Karen Moore
3 Baron-Hay Court
South Perth WA 6151

Acknowledgements

This project is supported by funding from Australian Pork Limited and the Department of Agriculture and Water Resources.

The technical assistance of Linley Valley Pork, DAFWA's Pork Innovation Group and Medina Research Station is gratefully acknowledged.

This research has been facilitated by access to Australian Proteome Analysis Facility which is funded by an initiative of the Australian Government as part of the National Collaborative Research Infrastructure Strategy.

Executive Summary

Immunisation of entire male pigs against gonadotrophin releasing factor (GnRF) is associated with increased feed intake and growth rate but there is also an increase in backfat compared to entire males. A way to reduce the increase in feed intake and increase in fat in immunocastrated (IC) male pigs is to restrict feed intake as IC male pigs that have been restrictively fed have been found to have a reduced backfat (Quiniou *et al.* 2012) and increased carcass leanness (Batorek *et al.* 2012). This project included two experiments that were designed to investigate the use of in-feed ingredients such as *Lupinus albus* (albus lupins) and a combination of calcium chloride and sodium tri-phosphate (mineral salts) to suppress the voluntary feed intake of IC male pigs when fed *ad libitum*.

The first experiment was a 2×3 factorial comprising two sexes (entire males and IC males) and three dietary treatments (control, 3% calcium chloride + 1.6% sodium tripolyphosphate, or 200-300 g/kg albus lupins. Immunocastrated male pigs fed the mineral salt diet ate less feed than those fed the control diet (2.92 vs. 3.16 kg/day) with no effect on growth rate. Immunocastrated male pigs fed the albus lupin diet had both a reduced feed intake and growth rate compared to those fed the standard finisher diet (2.30 vs. 3.16 kg/d and 0.86 vs. 1.15 kg/d for feed intake and growth performance, respectively). However, for the last 14 day period post-slaughter IC males fed the albus lupin diet at an inclusion rate of 20% albus lupins had a similar growth rate compared to entire males fed a standard finisher diet (1.05 vs. 1.09 kg/d) and feed intake (3.03 vs. 3.05 kg/d). The P2 backfat of IC males fed the albus lupin diet was also lower than control males receiving the standard finisher diet (8.71 vs. 9.34 mm). In conclusion, albus lupins show potential in reducing voluntary feed intake and thus reducing the increase in backfat associated with IC males, although growth rate was adversely affected. Mineral salts may be useful in situations where a reduction in feed intake and improvement in feed conversion is desired and reducing back fat is not the objective.

The second experiment was also a 2×3 factorial, comprising two sexes (entire males and IC males) and three dietary treatments (control, 200 g/kg albus lupins for the last 28 days prior to slaughter (Albus28) or only for the last 14 days (Albus14)). The inclusion of albus lupins at 20% in the diets of IC male pigs was successful at reducing feed intake, body fat and backfat to similar levels of entire males. However, the growth rate of the IC male pigs during the last 28 days of growth was adversely impacted by approximately 15% which is more than would be desirable in commercial pork production. In comparison, in Experiment 1 when albus lupins where included at 20% for the last 14 days pre-slaughter the feed intake, growth rate and backfat of IC males were similar to that of entire males. Due to the inconsistent results of the albus lupin response in reducing voluntary feed intake and subsequently reducing the growth rate of IC males, further investigation on the effect of albus lupins in the diets of these pigs is warranted. It is suggested that the effect of albus lupins on growth performance and backfat be further investigated using titrated levels of albus lupins, for example, from 10% to 20% to determine the appropriate level to optimise the decrease in feed intake and fat deposition whilst minimising the effect on growth rate.

Table of Contents

List of Tables

List of Figures

1. Background to Research

Immunisation of entire males against gonadotrophin releasing factor (GnRF) is associated with increased feed intake and growth rate but there is also an increase in backfat compared to entire males. A way to reduce the increase in feed intake and increase in fat in immunocastrated (IC) male pigs is to restrict feed intake as IC male pigs that have been restrictively fed have been found to have a reduced backfat (Quiniou *et al.* 2012) and increased carcass leanness (Batorek *et al.* 2012). This project aimed to use in-feed ingredients such as *Lupinus albus* (albus lupins) and a combination of calcium chloride and sodium tri-phosphate (mineral salts) to suppress the voluntary feed intake of immunised male pigs when fed *ad libitum*.

The aim of this project was to develop a dietary management strategy using albus lupins to reduce the increase in feed intake and subsequent increase in carcass fatness in immunocastrated male pigs.

2. Objectives of the Research Project

These experiments aimed to establish feeding strategies involving the inclusion of certain dietary ingredients that reduce voluntary fed intake of pigs, to improve production efficiency and reduce backfat and fat deposition in entire males immunised against GnRF. It also aimed to provide further information on the effectiveness of these feeding strategies in entire male pigs.

3. Experiment 1Introductory Technical Information

Immunisation of entire males against gonadotrophin releasing factor (GnRF) is an effective strategy to eliminate boar taint and a welfare friendly alternative to physical castration (Dunshea, 2009). The vaccine (Improvac®) is administered in two doses, one at approximately 10 weeks of age and the other approximately four weeks before slaughter. This allows the pig to grow as an entire male with associated positive effects on growth and carcass leanness. After the second vaccination any taint substances present are progressively metabolised (Dunshea *et al.* 2001a). However, after the second vaccination the pig has an increased feed intake and growth rate but there is also an increase in backfat compared to entire males (Dunshea *et al.* 2001a; Cronin *et al.* 2003; McCauley *et al.* 2003; Oliver *et al.* 2003; Lealiifano *et al.* 2011). In addition, body composition measures using dual-energy X-ray absorptiometry (DXA) have shown that the majority of fat deposition in pigs receiving their second dose of Improvac® at 80 kg live weight occurs 2 to 3 weeks after the second dose of Improvac® (Moore unpub, Pork CRC project 3A-101). Therefore, management strategies to limit this fat deposition and promote lean deposition are required.

A number of factors were considered which could potentially limit fat deposition and/or restrict feed intake in IC male pigs including porcine somatotrophin (pST; McCauley *et al.* 2003), ractopamine (Moore *et al.* 2009, Rikard-Bell *et al.* 2009), restricting feed intake (Batorek *et al.* 2012, Quiniou et al. 2012), low energy diet (Zeng *et al.* 2002), conjugated linoleic acid (Ostrowska *et al.* 1999, Dugan *et al.* 2004), and high fibre diets (McCauley *et al.* 2003). However, these were discounted due to market access issues (pST and ractopamine), potential welfare issues (restricting feed intake by restricting the amount of feed), on-farm practicability (pST and low energy diet) and cost (conjugated linoleic acid).

A way to reduce the increase in feed intake and increase in fat in IC male pigs is to restrict feed intake as IC male pigs that have been restrictively fed have been found to have a reduced backfat (Quiniou *et al.* 2012) and increased carcass leanness (Batorek *et al.* 2012). However as mentioned above restricting feed intake by restricting the amount of feed has welfare issues in terms of increased aggression (Batorek *et al.* 2012). Therefore if we can identify feeding strategies that reduce feed intake of IC males to similar levels to that of entire males (approximately 15% reduction) then this could become a sound management strategy.

This project aimed to use in-feed ingredients such as *Lupinus albus* (albus lupins) and a combination of calcium chloride and sodium tri-phosphate to suppress the voluntary feed intake of IC male pigs when fed *ad libitum*. *Lupinus albus* have been found to reduce feed intake in several pig experiments (Dunshea *et al.* 2001, Van Nevel *et al.* 2000). Dunshea *et al.* (2001b) suggests that the most likely mechanism by which albus lupins affects feed intake is by delayed transit in the stomach and small intestine. This delayed transit then feedbacks on satiety signals. A combination of calcium chloride and sodium tri-phosphate (mineral salts) has also been found to reduce feed intake in the range of 6 to 15% (Yen *et al.* 1981; Pluske *et al.* 2015). It suppresses appetite by changing the acid to base ratio by increasing the plasma chloride level and subsequently creating a HCO3- deficiency (Yen *et al.* 1981).

The hypotheses were:

1. Pigs immunised against GnRF which are fed either a diet containing *Lupinus albus* or mineral salts will have a reduced feed intake with no effect on growth rate compared to pigs receiving a standard finisher diet.

2. Pigs fed either *Lupinus albus* or mineral salts will deposit less fat compared to pigs receiving a standard finisher diet.
3. Pigs immunised against GnRF and fed either a diet containing *Lupinus albus* or mineral salts will have a similar backfat compared to entire males receiving a standard finisher diet.
4. Feeding pigs a diet containing *Lupinus albus* or mineral salts will have no effect on objective meat quality compared to a standard finisher diet.

4. Experiment 1 Research Methodology

The experimental protocol used was approved by the Department of Agriculture and Food Western Australia's Animal Research Committee and by the Animal Ethics Committee (Activity number 1-15-02). The animals were handled according to the Australian code of practice for the care and use of animals for scientific purposes (NHMRC, 2013).

A total of 294 Large White × Landrace × Duroc entire male and immunocastrated male pigs were used in this experiment. The experiment was a 2 × 3 factorial with the main treatments being: i) sex (entire males or IC males); and ii) feed ingredient (diet; control, 3% calcium chloride + 1.6% sodium tripolyphosphate, or 300 g/kg *Lupinus albus* (albus lupins)).

4.1 Allocation and housing

Two hundred and ninety four entire male pigs were sourced from a high health status commercial herd at 34.9 ± 3.98 kg (mean ± SD) liveweight (LW). Upon arrival the pigs were individually identified with ear tags, weighed and stratified on their LW. The allocated pigs received a priming dose of anti-gonadotrophin releasing factor immunological product (Improvac®, Zoetis Australia, Rhodes, Australia) on d -28 (where d 0 is when all pigs received the second dose of the anti-gonadotrophin releasing factor vaccine). The pigs were group housed (n=7) in a naturally ventilated grower shed. They had *ad libitum* access to water, and a commercial feed via a single spaced feeder.

4.2 Diets and feeding regime

On d 0 all pigs received the experimental diet and the second dose of the anti-gonadotrophin releasing factor vaccine was given to the pigs who had received the priming dose. The entire males did not receive a placebo injection. The experimental diets were formulated to the same nutrient specifications (14 MJ DE and 0.64 g standard ileal digestible lysine (SID)/MJ DE (high) or 0.50 g SID/MJ DE (low)). The diets were formulated so that the IC male pigs were fed as entire males for 2 weeks (from Day 0; high) and then the lysine level in the diet was reduced for the remaining 2 weeks (low; based on recommendations from Moore *et al.* 2016). The entire male pigs continued to receive the diet adequate for an entire male pig (high). The composition of the experimental diets is given in Table 1. The diets were also analysed for quantitative AA composition (Australian Proteome Analysis Facility, Sydney, NSW, Australia) and the results are presented in Table 2.

Table 1: Composition of the experimental diet

Ingredients g/kg, as-fed	Control low	Control high	Albus low	Albus high	Mineral salts low	Mineral salts high
Barley	400	400	400	400	400	400
Wheat	372	181	170	172	299	190
Mill run, 15%	50	45.7	82.3	48.5	20.0	24.6
Lupins, 28%	100	150	0	0	60	100
Lupins albus	0	0	300	300	0	0
Canola meal, 36%	0	150	0	0	100	150
Soybean meal, 48%	10	10	0	19.5	10	10
Bloodmeal, 85%	12.6	8.81	0	20	4.15	19.1
Tallow	36.0	38.4	30.4	21.9	55	55
Limestone	12.2	10.3	11.3	11.1	0	0
Salt	2.00	2.00	2.00	2.00	2.00	2.00
L-Lysine HCL	2.13	2.12	0.22	0.42	1.98	1.96
Methionine	0.61	0.51	0.16	1.01	0	0.51
Phytase[1]	0.20	0.20	0.20	0.20	0.20	0.20
Choline chloride, 60%	1.24	0	2.05	1.93	0.47	0
Calcium chloride, 77%[2]	0	0	0	0	16.0	16.0
Sodium tripolyphosphate[3]	0	0	0	0	3.00	3.00
Vitamins and minerals[4]	1.00	1.00	1.00	1.00	1.00	1.00
Nutrient Composition[5]						
DE, MJ/kg	14.0	14.0	14.0	14.0	14.0	14.0
NE, MJ/kg	9.98	9.45	9.24	9.31	10.1	9.69
CP, g/kg	145	188	184	207	150	181
Ca, g/kg	8.00	8.00	8.00	8.00	12.2	12.5
Total P, g/kg	5.92	6.83	6.40	6.24	10.2	10.6
Available P, g/kg	4.22	4.50	4.50	4.50	7.74	7.86
Na, g/kg	1.09	1.15	0.96	1.10	6.02	6.18
NDF, g/kg	16.8	19.7	18.9	17.8	16.6	17.8
ADF, g/kg	5.30	5.63	7.00	7.10	4.34	4.74
g SID Lys/MJ DE[6]	0.50	0.64	0.50	0.64	0.50	0.64

[1]Phytase from Phyzyme, Danisco Australia Pty Ltd.

[2]Calcium chloride dihydrate, Redox Pty Ltd Bibra Lake WA Australia.

[3]Sodium tripolyphosphate STPP FCC Low Nitrate/Nitrite, Redox Pty Ltd Bibra Lake WA Australia.

[4]Provided per kg of final diet: 7000 IU Vitamin A, 1400 IU Vitamin D3, 20 g Vitamin E, 1 g Vitamin K, 1 g Vitamin B1, 3 g Vitamin B2, 1.5 g Vitamin B6, 15 mg Vitamin B12 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.

[5]Calculated composition.

[6]SID: standardised ileal digestible lysine/MJ digestible energy.

Table 2: Quantitative amino acid analysis of the diets.

Amino acid g/kg, as-fed	Control low	Control high	Albus low	Albus high	Mineral salts low	Mineral salts high
Histidine	3.9	4.4	3.8	4.9	3.5	5.0
Isoleucine	5.1	6.0	6.2	6.7	5.1	5.9
Leucine	10.3	11.5	11.2	14.0	9.6	12.9
Lysine	8.1	9.5	7.4	9.4	7.7	10.3
Methionine	2.6	2.5	1.6	2.2	1.9	1.2
Phenylalanine	6.5	7.1	6.8	8.3	6.1	7.8
Threonine	5.5	6.3	5.8	6.9	5.2	6.7
Valine	7.4	8.3	7.4	9.2	6.9	9.2
Alanine	6.4	7.0	6.1	7.5	5.9	7.6
Arginine	9.4	10.8	12.9	14.8	8.2	10.8
Aspartic acid	10.9	12.4	13.4	15.9	10.0	13.3
Glycine	7.1	7.8	7.0	7.8	6.6	7.6
Glutamic acid	27.8	30.4	32.1	35.0	26.5	30.9
Proline	9.6	10.2	9.5	10.2	9.5	10.2
Serine	6.7	7.5	7.7	9.0	6.1	7.9
Tyrosine	3.4	3.8	4.2	4.8	3.0	3.6

4.3 Growth performance

Pigs were weighed weekly and feed intake determined on d 0, 7, 14, 21 and 28 to measure average daily gain and voluntary feed intake. The feed conversion ratio was calculated on a weekly basis from when the feeding of the experimental diets commenced.

4.4 Dual-energy X-ray Absorptiometry analysis

Twelve pigs per treatment (3 pigs/pen randomly selected from 4 replicate pens, so 72 in total (12 pigs x 6 treatments)) were scanned on d -1, 13 and 27 using dual-energy x-ray absorptiometry (DXA). The pigs were removed from feed and fasted for approximately 16 hr before scanning. Immediately before 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 BW. When sufficiently sedated the pigs were transferred to the DXA machine (Norland XR46 Densitometer Machine) (Suster *et al.* 2006). 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 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.

4.5 Slaughter procedure

Four weeks after the diets were introduced the pigs were individually tattooed, removed from feed overnight and transported to a commercial abattoir (approximately 90 min transport time). The pigs were stunned using a carbon dioxide, dip-lift stunner set at 85% CO_2 for 1.8 min (Butina, Denmark). Exsanguination, scalding, dehairing and evisceration were performed using standard commercial procedures. Hot carcass weight (HCW, 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 min after exsanguination, prior to chiller entry (2◦C, airspeed 4 m/sec).

4.6 Blood analysis

Blood samples (20 mL in lithium heparin tubes) were collected on d 0, 7, 14, 21 and 28 from the same pigs that were scanned with the DXA machine. The blood samples were centrifuged at 2,000 g for 15 min to recover plasma and were stored at -20°C until analysed. Plasma urea nitrogen (PUN) was quantified using a commercial kit (Beckman Coulter/Olympus Reagent Kit Cat. No. OSR6134 Lot #6042). Plasma urea (mmol/L) was converted to PUN (mg/dL) by dividing by 0.357. Calcium was quantified using a commercial kit (Beckman Coulter/Olympus Reagent Kit Cat. No. OSR60117 Lot #6564). Phosphorus was quantified using a commercial kit (Beckman Coulter/Olympus Reagent Kit Cat. No. OSR6122 Lot #5797). Glucose was quantified using a commercial kit (Beckman Coulter/Olympus Reagent Kit Cat. No. OSR6121 Lot #5512). The assays for PUN, calcium, phosphorus and glucose were performed on an automated analyser according to the manufacturer's instructions (Olympus AU400; Olympus UK Ltd, Hertfordshire, United Kingdom). Plasma leptin was determined using a commercial kit (Cusabio Pig Leptin Elisa (CSB-E06815p, Jomar Life Research Pty Ltd, VIC, Australia). Sodium, chloride and total carbon dioxide were determined and the assays were performed on an automated analyser (AU680, Beckman Coulter).

4.7 Objective meat quality

pH and temperature decline in the *Longissimus thoracis* (LT) was measured at 45 mins 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. The pH meter was calibrated on two standards (pH 4.01 and 7.0) as per the manufacturer's instructions. At 24 hours post-slaughter a section of the LT muscle was removed from the left hand side of the carcass between the 12th and 13th rib. For determination of pH and temperature a 2 cm steak was cut from the appropriate sample and measured using the pH/temperature meter as previously described. 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 minutes. An 80 ± 5 g sample was cut from the loin samples to measure cooking loss and shear force (Bouton *et al*., 1971). The samples were 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 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).

4.8 Statistical analysis

General analysis of variance was performed with the GENSTAT 16 program (VSN International Ltd, Hemel Hempstead, UK) to analyse the main effects of sex and diet on growth performance, carcass quality, body composition and physiological measures. Batch was used as a block in the analysis. Pen was used as the experimental unit for the growth performance and carcass quality. Pig was used as the experimental unit for body composition and physiological measures. Repeated measures were used to analyse the physiological measures. A level of probability of less than 0.05 was used to determine statistical difference between the means. A level of probability of less than 0.1 but greater than 0.05 was determined to be a trend. Fisher's-protected least significant differences were used to determine differences among treatments.

5. Experiment 1Results

5.1 Growth and carcass performance

The growth and carcass performance results are presented in Table 3. Average daily gain was not affected by sex from d 0-14 ($P>0.05$). However, from d 15-28 and d 0-28 IC males grew faster compared to entire males ($P<0.001$ and $P<0.001$, respectively). Immunocastrated males had an increased feed intake for d 0-14, 15-28 and 0-28 ($P=0.043$, $P<0.001$ and $P<0.001$, respectively). The feed conversion ratio was not affected by sex from d 0-14 ($P=0.979$). However, from d 15-28 and d 0-28 IC males had a worse feed conversion ratio compared to entire males ($P<0.001$ and $P<0.001$, respectively). There was a trend for IC males to have a heavier carcass weight compared to entire males ($P=0.063$). Immunocastrated males had a lower dressing percentage ($P<0.001$) and a higher P2 backfat ($P<0.001$) compared to entire males.

Pigs fed albus lupins grew more slowly compared to those fed the control or mineral salt diet for d 0-14, d 15-28 and d 0-28 ($P<0.001$, $P<0.001$ and $P<0.001$, respectively). There was no difference in average daily gain between the control diet and mineral salt diet. Pigs fed albus lupins ate less feed than those on the mineral salt diet who in turn ate less than those on the control diet for all time periods ($P<0.001$, $P<0.001$ and $P<0.001$ for d 0-14, d 15-28 and d 0-28, respectively). From d 0-14 pigs fed the mineral salt diet had a better feed conversion compared to the other diets ($P=0.049$). From d 15-28 pigs fed the albus lupin diet and the mineral salt diet had a better feed conversion than those fed the control diet ($P=0.007$). From d 0-28 pigs fed the mineral salt diet had a better feed conversion than those on the albus lupin diet who in turn had a better feed conversion than the control diet ($P=0.001$). Pigs fed the albus lupin diet had a lower carcass weight ($P<0.001$), lower dressing percentage ($P=0.008$) and a lower P2 backfat ($P<0.001$) compared to those on the mineral salt and control diet.

There were no interactions ($P>0.05$) between sex and feed for any growth or carcass measurement.

Table 3: Growth and carcass performance for entire male and immunocastrated male pigs fed three different diets from 67.5 to 95.4 kg LW (n=7).

	Entire male			Immunocastrated male			SED[a]	P-value		
	Control	Mineral salt	Albus lupin	Control	Mineral salt	Albus lupin		Sex	Diet	S×D
Daily gain (kg/day)										
d 0-14	1.00	0.984	0.648	1.07	1.00	0.673	0.037	0.106	<0.001	0.665
d 15-28	1.09	1.06	0.930	1.23	1.25	1.050	0.036	<0.001	<0.001	0.376
d 0-28	1.05	1.02	0.789	1.15	1.13	0.861	0.026	<0.001	<0.001	0.633
Feed intake (kg/day)										
d 0-14	2.52	2.25	1.61	2.63	2.39	1.64	0.076	0.043	<0.001	0.585
d 15-28	3.05	2.80	2.47	3.80	3.58	3.03	0.090	<0.001	<0.001	0.162
d 0-28	2.72	2.48	1.98	3.16	2.92	2.30	0.075	<0.001	<0.001	0.456
Feed conversion ratio										
d 0-14	2.52	2.29	2.51	2.48	2.39	2.45	0.094	0.979	0.049	0.448
d 15-28	2.81	2.64	2.67	3.10	2.86	2.90	0.091	<0.001	0.007	0.821
d 0-28	2.60	2.43	2.52	2.75	2.59	2.67	0.058	<0.001	0.001	0.985
CW (kg)	64.7	64.3	58.8	65.6	65.2	59.8	0.820	0.063	<0.001	0.996
DP (%)	66.8	66.9	65.7	65.9	65.6	65.1	0.428	<0.001	0.008	0.402
P2 backfat (mm)[b]	9.34	8.83	7.49	10.8	10.7	8.71	0.508	<0.001	0.005	0.432

[a] SED for Sex×Diet

[b] Carcass weight used as a covariate.

5.2 Body composition

The body composition results are presented in Table 4. Bone mass composition was increased for IC males compared to entire males for d -1-13 (P=0.035) and decreased from d 14-27 (P=0.018). There was no difference between IC males and entire males for bone mass composition from d -1-27 (P=0.984). There was no difference (P>0.05) in lean deposition between IC males and entire males for all time periods. There was no difference (P>0.05) in fat deposition between IC males and entire males for d -1-13. Immunocastrated males deposited more fat than entire males from d 14-27 (P<0.001) and from d -1-27 (P<0.001). Immunocastrated males also had an increased g fat per kg metabolic body weight from d 14-27 (P=0.027) and d -1-27 (P<0.001) compared to entire males. There was no difference (P>0.05) between sexes from d -1-13.

Pigs fed albus lupins had a lower bone mineral content than those fed a control or mineral salt diet from d -1-13 (P=0.003). Pigs fed the mineral salt diet had a higher bone mineral content than those fed a control or albus lupin diet from d 14-27 (P=0.003). From d -1-27 pigs fed albus lupins had a lower bone mineral content compared to those fed a control diet which in turn was lower than those fed the mineral salt diet (P<0.001). Pigs fed albus lupins deposited less lean from d -1-13 (P<0.001) and d 1-27 (P<0.001) than those on a control or mineral salt diet. There was no difference

between diets (P>0.05) for d 14-27. Pigs fed albus lupins deposited less fat and had a lower g fat per kg metabolic body weight from d -1-13 (P<0.001 and P<0.001, respectively), d 14-27 (P<0.001 and P=0.027, respectively) and d -1-27 (P<0.001 and P<0.001, respectively) than those on a control or mineral salt diet.

There were no interactions (P>0.05) between sex and feed for any body composition measurement.

Table 4: Body composition for entire male and immunocastrated male pigs fed three different diets from 67.5 to 95.4 kg LW (n=12).

	Entire male			Immunocastrated male			SED[a]	P-value		
	Control	Mineral salt	Albus lupin	Control	Mineral salt	Albus lupin		Sex	Diet	S×D
BMC[b] (kg/day)										
d -1-13	22.0	20.6	13.4	23.3	23.1	16.9	1.94	0.035	<0.001	0.728
d 14-27	18.1	23.9	20.5	16.5	20.5	17.1	2.00	0.018	0.003	0.761
d -1-27	20.2	22.3	16.9	19.6	22.7	17.0	1.39	0.984	<0.001	0.880
Lean (g/day)										
d -1-13	736	709	499	783	694	525	43.2	0.446	<0.001	0.589
d 14-27	706	765	752	737	762	626	52.5	0.289	0.140	0.096
d -1-27	725	737	625	758	728	576	34.1	0.668	<0.001	0.236
Fat (g/day)										
d -1-13	189	196	57.6	202	173	97.9	30.9	0.571	<0.001	0.353
d 14-27	238	294	166	465	487	369	38.5	<0.001	<0.001	0.815
d -1-27	222	245	112	321	330	234	23.2	<0.001	<0.001	0.527
g Fat/kg Metabolic BW										
d -1-13	423	399	160	405	350	261	70.6	0.782	<0.001	0.291
d 14-27	445	551	367	770	788	712	66.0	<0.001	0.027	0.474
d -1-27	266	281	170	344	353	304	25.6	<0.001	<0.001	0.177

[a] SED for Sex × Diet

[b] BMC – bone mineral content

5.3 Physiological measures

There was a trend for IC males to have a higher PUN concentration than entire males (p = 0.073, Figure 1). Pigs fed the mineral salt diet had a lower PUN concentration than those fed the control which in turn was lower than those fed the albus lupin diet (P<0.001). Plasma urea nitrogen concentration varied with time. In general it was lower on d 7 and d 21 compared to the other days (p < 0.001). There was a time by sex interaction (p = 0.002) for PUN concentration with PUN concentration on d 21 for entire males being less than IC males on d 21. There was a time by feed interaction in that pigs fed the mineral salt diet had a lower PUN concentration on d 7 and 14 compared to those on the control and albus lupin diets (p < 0.001). There was also a time by sex by feed interaction (p = 0.022).

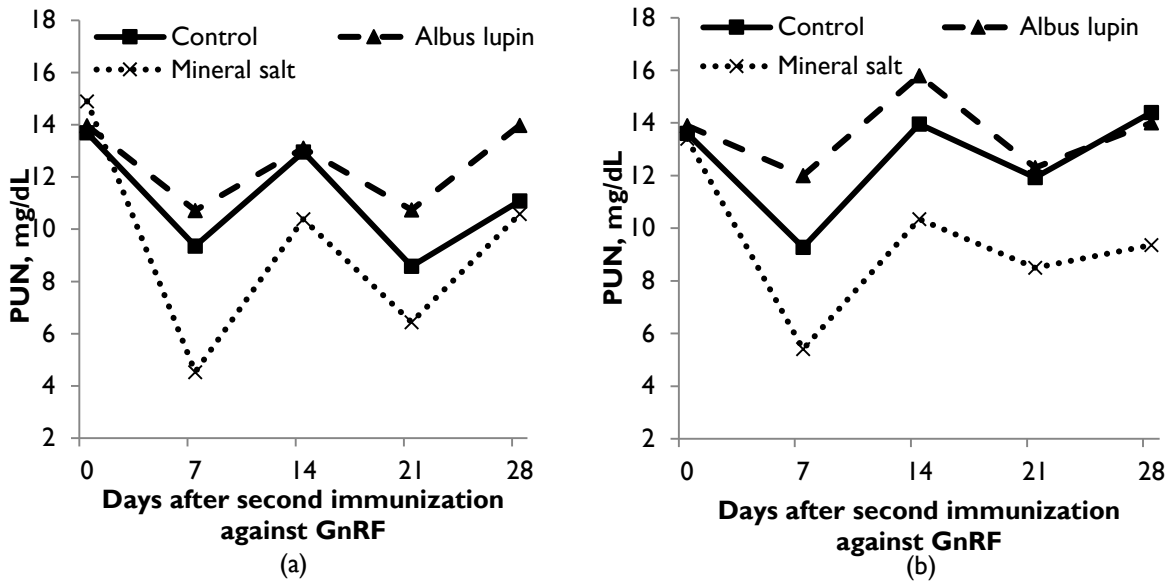

Figure 1: Change in plasma urea nitrogen for a) entire males and b) immunocastrated male pigs fed three different diets for 28 days after the second immunisation against gonadotrophin releasing factor (n=12).

There was no effect of sex (P=0.946) or diet (P=0.908) however glucose decreased over time (P<0.001; Figure 2). There were no interactions between sex, diet and time for glucose.

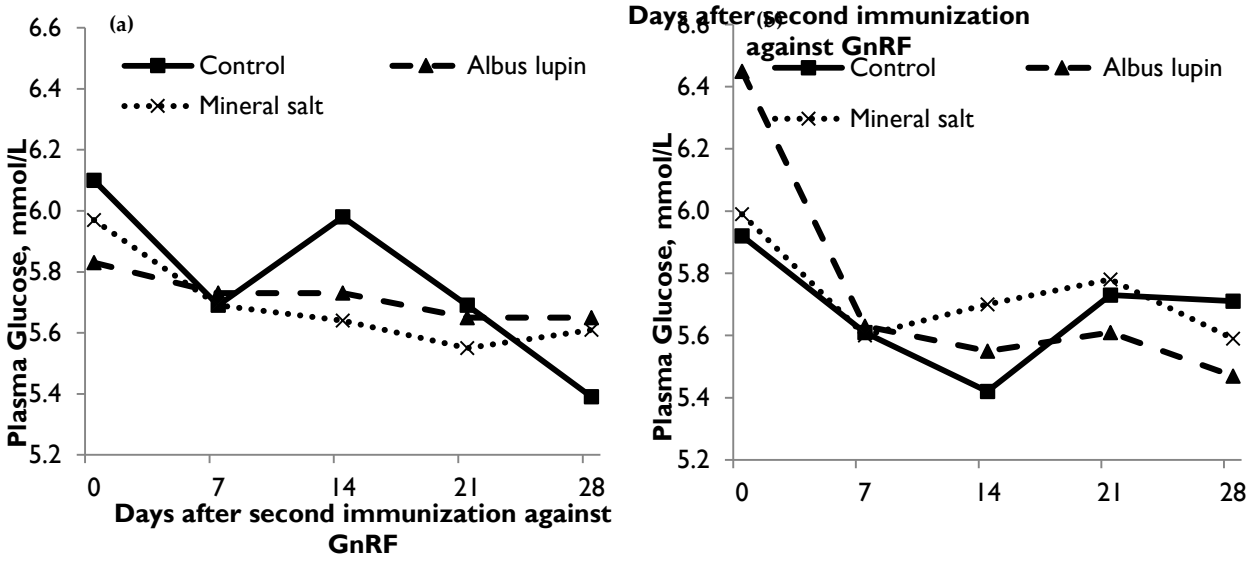

Figure 2: Change in glucose for a) entire males and b) immunocastrated male pigs fed three different diets for 28 days after the second immunisation against gonadotrophin releasing factor (n=12).

Entire male pigs had a lower phosphate concentration compared to the IC males (P=0.026, Figure 3). Pigs fed the mineral salt diet had a higher phosphate concentration compared to those fed the control and albus lupin diet (P<0.001). The phosphate concentration was higher on d 0 compared to the other days (P<0.001). There was a time by diet interaction where pigs fed the mineral salt diet had a higher phosphate concentration compared to the albus lupin and control diet from d 7 onwards (P<0.001; Figure 3).

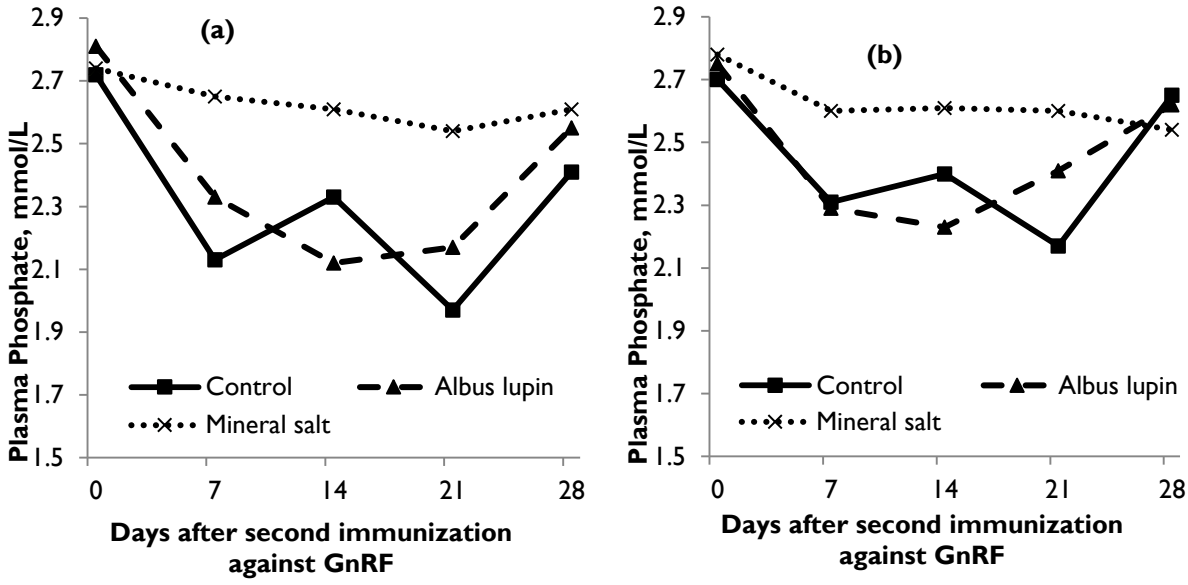

Figure 3: Change in phosphate for a) entire males and b) immunocastrated male pigs fed three different diets for 28 days after the second immunisation against gonadotrophin releasing factor (n=12).

Entire males had a lower plasma chloride concentrations than IC males (P=0.039); Figure 4). Pigs fed the mineral salt diet had an increased chloride concentration (P<0.001 for time × diet interaction) from d 7 onwards compared to those fed the control or albus lupin diet. There were no other interactions (P>0.05).

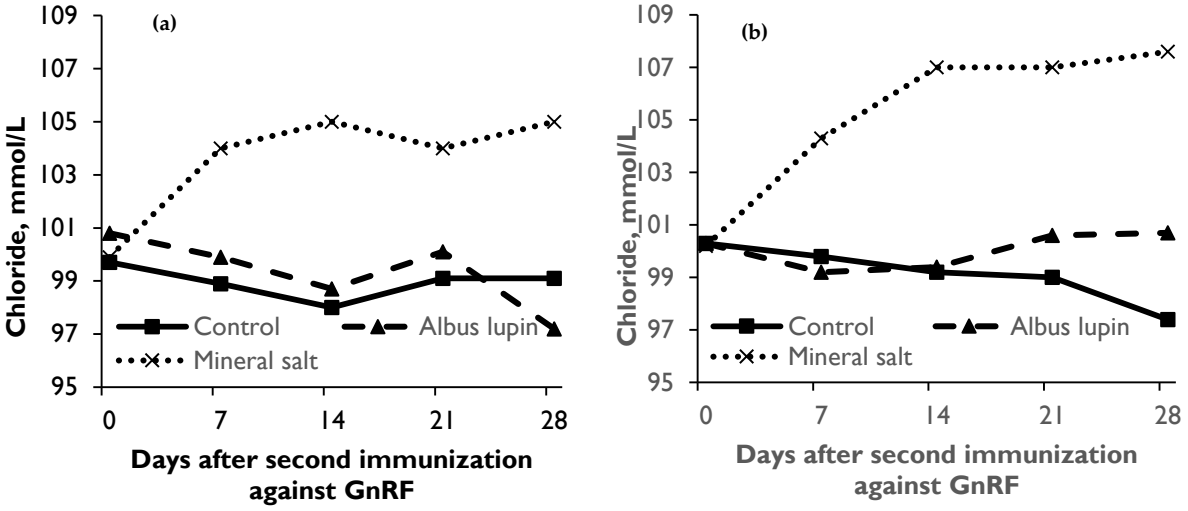

Figure 4: Change in chloride for a) entire males and b) immunocastrated male pigs fed three different diets for 28 days after the second immunisation against gonadotrophin releasing factor (n=12).

There was no effect of sex on calcium concentration (P>0.05, Figure 5). Pigs fed the control diet had a higher calcium concentration than those on the albus diet who in turn had a higher calcium concentration than those on the mineral salt diet (P<0.001). Calcium concentration was lower on d 0 compared to the other time periods (P<0.001). There was a time by sex by feed interaction in that IC

male pigs fed the albus lupin and mineral salt diet had a lower calcium concentration from d 7 onwards compared to the control diet, however, only the entire males on the mineral salt diet appeared to have a lower calcium concentration from d 14 onwards. (P=0.011; Figure 5).

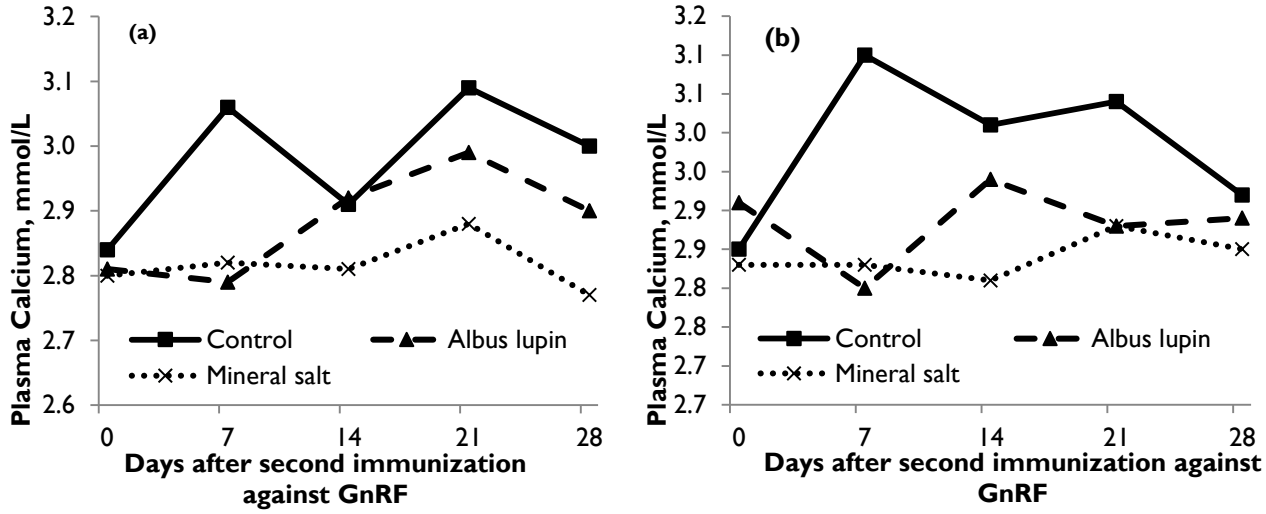

Figure 5: Change in calcium for a) entire males and b) immunocastrated male pigs fed three different diets for 28 days after the second immunisation against gonadotrophin releasing factor (n=12).

There was no difference in sodium concentration between sex (P>0.05), or diet (P>0.05) and there were no interactions (P>0.05; Figure 6). However, there was a time effect in that the sodium concentration was lower (P=0.034) on d 0 compared to d 14, d 21 and d 28. There was no difference in sodium concentration between d 0 and d 7.

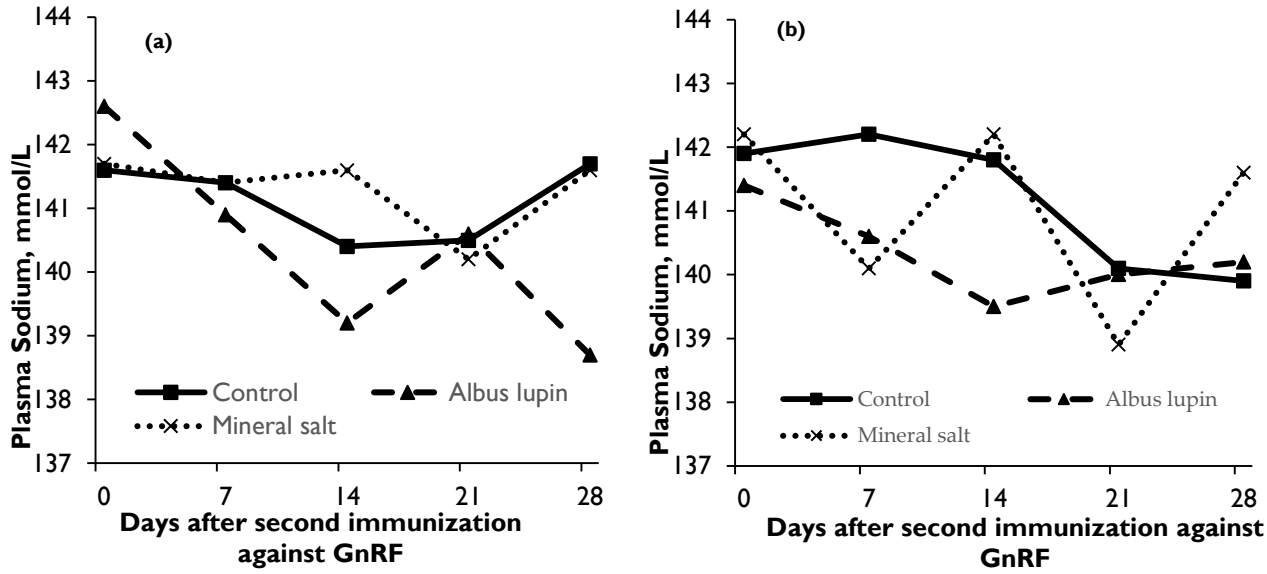

Figure 6: Change in sodium for a) entire males and b) immunocastrated male pigs fed three different diets for 28 days after the second immunisation against gonadotrophin releasing factor (n=12).

There was a trend for entire males to have a higher carbon dioxide concentration than IC males (p = 0.065; Figure 7). Pigs fed mineral salts had a lower carbon dioxide concentration compared to those fed the control or albus lupin diet (p < 0.001). There was a time by diet interaction (p < 0.001) in that pigs fed the mineral salt diet had a lower carbon dioxide concentration from d 7 onwards

compared to the other diets. There was also a time by sex interaction (p = 0.024) where on d 21 and d 28 IC males had a lower carbon dioxide concentration than entire males.

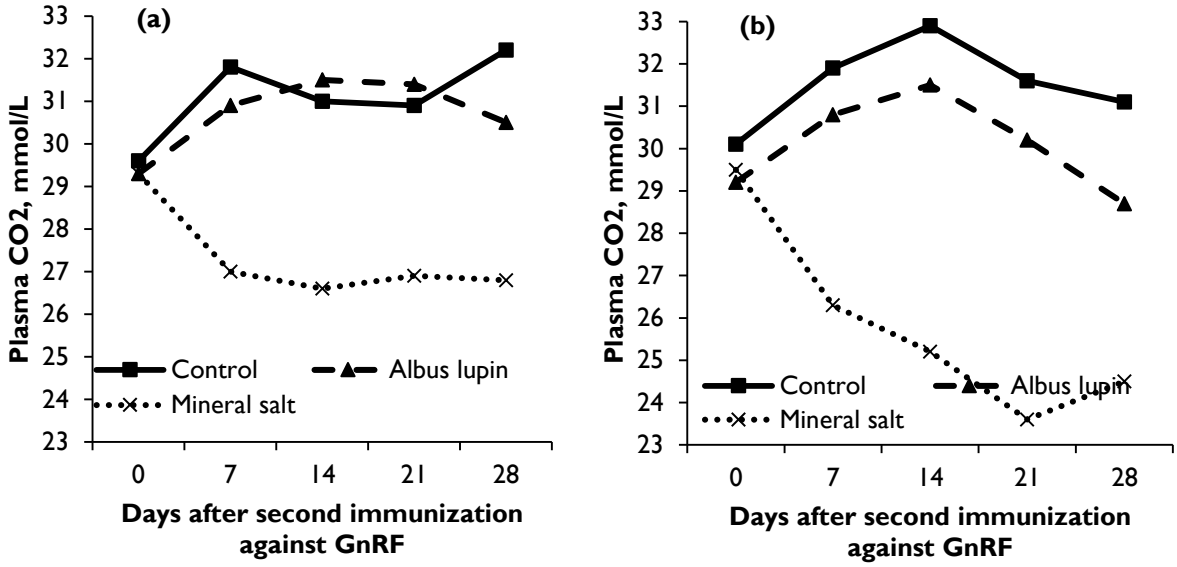

Figure 7: Change in carbon dioxide for a) entire males and b) immunocastrated male pigs fed three different diets for 28 days after the second immunisation against gonadotrophin releasing factor.

Entire males fed the albus lupin diet had lower plasma leptin compared to those on the control or mineral salt diet (p = 0.021). There was no difference between diets for IC males. There was a time by sex interaction for leptin where from d 7 onwards IC males had a lower leptin concentration compared to entire males (p < 0.001; Figure 8).There were no other effects or interactions between time, sex and diet for leptin concentration (p > 0.05).

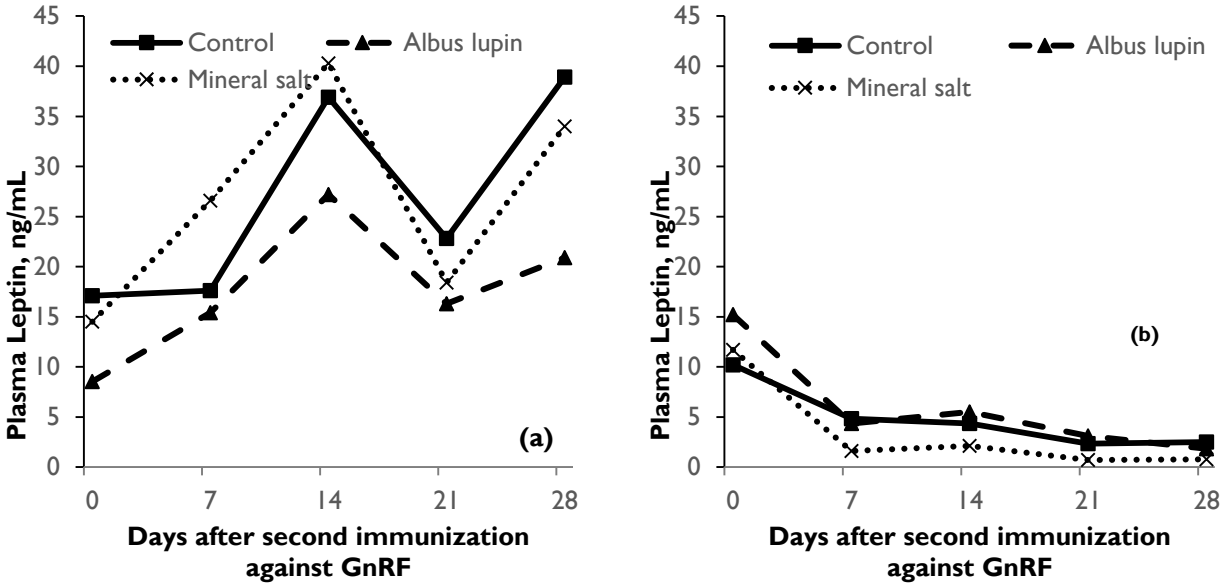

Figure 8: Change in leptin for a) entire males and b) immunocastrated male pigs fed three different diets for 28 days after the second immunisation against gonadotrophin releasing factor.

5.4 Objective meat quality

The objective meat quality results are given in Table 5. Pork from IC males had a higher ultimate pH (P<0.001), was lighter (L*; P=0.003), more yellow (P=0.008) and had a higher drip loss (P<0.001) compared to meat from entire males. There was no difference in relative redness, cook loss and shear force between IC males and entire males.

Pigs fed the mineral salt diet or an albus diet had a higher pH (P=0.046) at 45 minutes post-exsanguination compared to those fed the control diet. There was no difference (P>0.05) between diets for any other measure of objective meat quality. There were no interactions (P>0.05) between sex and diet.

Table 5: Objective meat quality for entire male and immunocastrated male pigs fed three different diets (n=21).

	Entire male			Immunocastrated male			SED[a]	P-value		
	Control	Mineral salt	Albus lupin	Control	Mineral salt	Albus lupin		Sex	Diet	S×D
pH 45 min	6.18	6.24	6.24	6.02	6.26	6.20	0.082	0.235	0.026	0.319
pH 24 hr	5.62	5.54	5.62	5.58	5.67	5.52	0.043	<0.001	0.812	0.218
L	49.4	48.7	48.5	50.8	50.4	51.1	1.10	0.003	0.771	0.687
a	5.82	5.56	5.08	6.01	5.31	6.04	0.377	0.172	0.174	0.076
b	4.15	3.91	3.51	4.54	4.16	4.64	0.377	0.008	0.457	0.215
Drip loss (%)	4.75	3.79	3.57	6.12	5.04	6.18	0.725	<0.001	0.144	0.342
Cook loss (%)[b]	24.5	23.6	23.6	21.9	23.1	25.8	1.14	0.735	0.355	0.124
Shear force (N)[b]	47.0	42.6	45.1	41.3	43.4	44.7	2.71	0.415	0.782	0.432

[a] SED for Sex×Diet

[b] For Batch 1 only (n=12). Batch 2 samples were accidentally aged.

6. Experiment 1Discussion

The hypothesis that pigs immunised against GnRF which were fed either a diet containing albus lupins or mineral salts would have a reduced feed intake with no effect on growth rate compared to pigs receiving a standard finisher diet was partially supported. Immunocastrated male pigs fed the mineral salt diet ate less feed than those fed the control diet with no effect on growth rate. However, IC male pigs fed the albus lupin diet had both a reduced feed intake and growth rate compared to those fed the standard finisher diet.

The results for the mineral salt diet are in contrast to Yen *et al.* (1981) and Pluske *et al.* (2015) who found pigs fed a mineral salt diet had both a lower daily feed intake (32 and 15%, respectively) and weight gain (45 and 15%, respectively) compared to those on the basal diet. However, Yen *et al.* (1981) fed 4% $CaCl_2.2H_2O$ and 2.22% $Na_5P_3O_{10}$ compared to the concentration of 3% $CaCl_2.2H_2O$ and 1.6% $Na_5P_3O_{10}$ in this study. Pluske *et al.* (2015) used 4% $CaCl_2$ and 2.2% $Na_5P_3O_{10}$ for one week before halving the concentration for the remaining two weeks due to acceptance issues of the diet in the first week. The differences in concentration levels between studies may help to explain the observed differences in performance. In addition, the acceptance issues in Pluske *et al.* (2015) may be because calcium chloride was used rather than calcium chloride dihydrate which increased the effective level of calcium chloride to 94% compared to 77% calcium chloride both in the current experiment and in Yen *et al.* (1981).

The most likely explanation by which $CaCl_2$ reduced feed intake is through the process of metabolic acidosis. Yen *et al.* (1981) found that the acidosis is caused by an increase in the plasma chloride level which sets off a number of events resulting in a reduction in the buffering capacity of the HCO^-_3. This then leads to a low blood pH and a reduction in total CO_2. We also found an increased plasma chloride concentration and a reduction in CO_2 however we did not measure blood pH or HCO^-_3. Although there was an increase in the dietary concentration of calcium this was not reflected in the plasma calcium concentrations because the calcium is excreted as $Ca_3(PO_4)_2$ in the faeces (Soine and Wilson, 1967 as cited by Yen *et al.* 1981).

The albus lupins were originally included in the diet at 30% based on findings from Dunshea *et al.* (2001) and Van Nevel *et al.* (2000). These researchers observed reductions in feed intake in the finishing period of between 12 and 27% (van Nevel *et al.* 2000 and Dunshea *et al.* 2000). We were trying to decrease the feed intake of the IC males to similar intake levels of entire males, a reduction of approximately 15% which corresponded with the previous findings of reductions in intake. However, in the current experiment from d 0-7 feed intake of the pigs receiving the albus lupin diet was nearly halved compared to the control diet. It is suggested that the observed differences in intake between the experiments may be because the pigs in the Dunshea *et al.* (2001) and van Nevel *et al.* (2000) experiments were acclimatised to the albus lupin diet before the experimental period. There was no acclimatisation to diet in the present study.

When the daily gain, feed intake and feed conversion ratio data were examined for the d 15-28 period only and the pigs were fed a diet with 20% albus lupins, the IC male pigs on the albus lupin diet had a similar daily gain (1.05 vs. 1.09 kg/d), feed intake (3.03 vs. 3.05 kg/d) and feed conversion (2.90 vs. 2.81) compared to entire males fed the control diet. Therefore, there is potential for albus lupins to reduce the feed intake of IC males, however, growth rate is also reduced to similar to that of entire males.

Albus lupins are thought to decrease feed intake by delayed transit through the stomach and small intestine. This may then feedback through satiety signals (Dunshea *et al.* 2001). This theory may be supported by the decreased dressing percentage observed in pigs fed albus lupins in both the current experiment and by other researchers (Dunshea *et al.* 2001, King 1981 and Van Nevel *et al.* 2000).

The hypothesis that pigs fed either albus lupins or mineral salts will deposit less fat compared to pigs receiving a standard finisher (control) diet was partially supported. Pigs fed albus lupins deposited less fat compared to those on both the control and mineral salt diet. There was no difference in fat deposition between pigs fed the control or mineral salt diet. As far as we are aware there is no previous research which has investigated using these in-feed ingredients to reduce feed intake with the main objective being to reduce fat deposition.

Given that there was a reduction in feed intake in pigs receiving the mineral salt diet it was anticipated that there would be a corresponding reduction in fat deposition. However, even though there was a reduction in feed intake of 9.2% for the IC males on the mineral salt diet compared to the control diet perhaps this was insufficient to promote a decrease in fat deposition. The IC male pigs fed the mineral salt diet still consumed 9.3% more feed than the entire males fed the control diet. When diets of IC male pigs have been restricted previously by restricting the amount of feed reductions in backfat have been observed when the feed intakes were between 15 and 22% lower than when fed *ad libitum* (Quiniou *et al.*, 2012).

The hypothesis that pigs immunised against GnRF and fed either a diet containing albus lupins or mineral salts would have a similar backfat compared to entire males receiving a standard finisher diet was partially supported. Immunocastrated male pigs fed the albus lupin diet had a similar backfat compared to entire males receiving the standard finisher diet. However, IC males fed the mineral salt diet were 1.36 mm fatter than entire males receiving the control diet and had a similar backfat to IC males receiving the control diet. Therefore, albus lupins show potential in reducing the increase in backfat associated with IC males although there was also a reduction in dressing percentage. Further investigation should be undertaken to determine the effect of including a constant 20% albus lupins in the diet of IC males for either 28 or 14 days pre-slaughter. Van Nevel *et al.* (2000) also found that including albus lupins in diets at 30% reduced backfat thickness with a tendency for the percentage of lean content to increase. The reduction in backfat thickness and increase in lean was attributed to the slower growth rates (van Nevel *et al.* 2000).

The hypothesis that feeding pigs a diet containing *Lupinus albus* or mineral salts will have no effect on objective meat quality compared to a standard finisher diet was supported. There was no difference in objective meat quality between any diets. When including alternative ingredients in pig diets it is important to ensure that there is no adverse impact on the meat quality of the diets. There does not appear to be any other research investigating the effect of including mineral salts in the diet on meat quality. Kim *et al.* (2011) investigated including *Lupinus angustifolou*s in pig diets at 350 g/kg and found no effect on meat quality. There does not appear to be any other work investigating the effect of albus lupins on meat quality in pigs.

7. Experiment 2 Introductory Technical Information

Experiment 1 evaluated two potential infeed ingredients (a mixture of mineral salts or 20-30% albus lupins *albus)* to reduce the feed intake of pigs immunised against GnRF. The inclusion of albus lupins appeared to have had some success in decreasing fat deposition in both entire males and immunised males. For example, at a similar carcass weight the back fat of immunised males receiving the albus lupin diet was less than the back fat of entire males receiving the control diet (8.7 vs. 9.3 cm). Further work is required to determine the effect of albus lupins on feed intake and fat deposition over the 4 weeks after the second immunisation of GnRF. It is also proposed to compare this to including albus lupins in the second two week period after the second immunisation against GnRF only, as this is when feed intake increases considerably and when the majority of fat deposition of immunised male pigs occurs.

Therefore the aim of this experiment was to develop a dietary management strategy using albus lupins to reduce the increase in feed intake and subsequent increase in carcass fatness in immunised male pigs.

The hypotheses were:

1. Pigs immunised against GnRF which are fed a diet containing albus lupins for either 14 or 28 days prior to slaughter will have a reduced feed intake and growth rate compared to pigs immunised against GnRF receiving fed a standard finisher diet.
2. Pigs fed albus lupins will have less fat compared to pigs receiving a standard finisher diet.
3. Pigs immunised against GnRF and fed a diet containing albus lupins for either 14 or 28 days prior to slaughter will have a similar backfat compared to entire males receiving a standard finisher diet.
4. Pigs immunised against GnRF and fed albus lupins for 28 days prior to slaughter will have a lower overall daily feed intake but a similar fat composition compared to pigs immunised against GnRF and fed albus lupins for 14 days prior to slaughter.

8. Experiment 2 Research Methodology

The experimental protocol used was approved by the Department of Agriculture and Food Western Australia's Animal Research Committee and by the Animal Ethics Committee (Activity number 15-5-17). The animals were handled according to the Australian code of practice for the care and use of animals for scientific purposes (NHMRC, 2013).

A total of 294 Large White × Landrace × Duroc entire male and immunocastrated male pigs were used in this experiment. The experiment was a 2 × 3 factorial with the main treatments being: i) sex (entire males or IC males); and ii) feeding strategy (control, 200 g/kg albus lupins; Albus 28) for 28 days prior to slaughter, 200 g/kg albus lupins for the last 14 days prior to slaughter (Albus 14).

8.1 Allocation and housing

Two hundred and ninety four entire male pigs were sourced from a high health status commercial herd at 40.5 (mean) liveweight (LW). Upon arrival the pigs were individually identified with ear tags, weighed and stratified on their LW. The allocated pigs received a priming dose of anti-gonadotrophin releasing factor immunological product (Improvac®, Zoetis Australia, Rhodes, Australia) on d -28 (where d 0 is when all pigs received the second dose of the anti-gonadotrophin releasing factor vaccine). The pigs were group housed (n=7) in a naturally ventilated grower shed. They had *ad libitum* access to water, and a commercial feed via a single spaced feeder.

8.2 Diets and feeding regime

On d 0 all pigs received the experimental diet and the second dose of the anti-gonadotrophin releasing factor vaccine was given to the pigs who had received the priming dose. The entire males did not receive a placebo injection. The experimental diets were formulated to the same nutrient specifications (13.5 MJ DE and 0.64 g standard ileal digestible lysine (SID)/MJ DE (high) or 0.50 g SID/MJ DE (low)). The diets were formulated so that the IC male pigs were fed as entire males for 2 weeks (from d 0; high) and then the lysine level in the diet was reduced for the remaining 2 weeks (low; based on recommendations from Moore *et al.* 2016). The entire male pigs continued to receive the diet adequate for an entire male pig (high). The composition of the experimental diets is given in Table 6. The diets were also analysed for quantitative AA composition (Australian Proteome Analysis Facility, Sydney, NSW, Australia) and the results are presented in Table 7.

Table 6: Composition of the experimental diet

Ingredients g/kg, as-fed	Control low	Control high	Albus low	Albus high
Barley	400	400	400	579
Wheat	384	257	260	101
Mill run, 15%	50	85	91	10
Lupins, 28%	100	100	0	0
Lupins albus	0	0	200	200
Canola meal, 36%	20	100	10	49
Soybean meal, 48%	10	10	10	14.5
Bloodmeal, 85%	1.28	15.2	2	20
Tallow	15.3	15.7	10	10
Limestone	11.1	11.1	11.5	11.0
DiCal Phosphorus	1.31	0	0	0
Salt	2	2	2.48	2
L-Lysine HCL	2.43	2.29	0.61	0.83
Methionine	0.51	0.56	0	0.70
Phytase[1]	0.20	0.20	0.20	0.20
Choline chloride, 60%	1.02	0.32	1.76	1.53
Vitamins and minerals[3]	1.0	1.0	1.0	1.0
Nutrient Composition[4]				
DE, MJ/kg	13.5	13.5	13.5	13.5
CP, g/kg	144	176	172	190
Ca, g/kg	8.00	8.00	8.00	8.00
Total P, g/kg	6.35	6.85	6.53	6.25
Available P, g/kg	4.50	4.50	4.49	4.50
NDF, g/kg	175	196	188	182
ADF, g/kg	53.3	49.5	59.2	64.8
g SID Lys/MJ DE[5]	0.50	0.64	0.50	0.64

[1]Phytase from Phyzyme, Danisco Australia Pty Ltd.

[2]Calcium chloride dihydrate, Redox Pty Ltd Bibra Lake WA Australia.

[3]Provided per kg of final diet: 7000 IU Vitamin A, 1400 IU Vitamin D3, 20 g Vitamin E, 1 g Vitamin K, 1 g Vitamin B1, 3 g Vitamin B2, 1.5 g Vitamin B6, 15 mg Vitamin B12 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.

[4]Calculated composition.

[5]SID: standardised ileal digestible lysine/MJ digestible energy.

Table 7: Quantitative amino acid analysis of the diets.

Amino acid g/kg, as-fed	Control low	Control high	Albus low	Albus high
Histidine	3.6	4.7	3.7	4.9
Isoleucine	5.6	6.4	6.2	6.9
Leucine	10.1	12.7	11.1	14.2
Lysine	7.4	9.2	7.2	9.6
Methionine	1.8	2.4	1.6	1.9
Phenylalanine	6.7	8.2	7.0	9.1
Threonine	5.1	6.5	5.7	7.1
Valine	7.0	9.3	7.6	10.2
Alanine	5.5	7.7	5.9	8.1
Arginine	9.5	10.8	11.9	12.3
Aspartic acid	10.5	12.5	12.4	14.8
Glycine	6.3	8.8	6.8	8.4
Glutamic acid	34.0	37.4	35.1	38.1
Proline	11.1	13.6	10.7	14.0
Serine	6.8	8.0	7.6	8.7
Tyrosine	2.8	3.5	3.7	4.0

8.3 Growth performance

Pigs were weighed weekly and feed intake determined on d 0, 7, 14, 21 and 28 to measure average daily gain and voluntary feed intake. The feed conversion ratio was calculated on a weekly basis from when the feeding of the experimental diets commenced.

8.4 Dual x-ray absorptiometry

Twelve pigs per treatment (3 pigs/pen randomly selected from 4 replicate pens, so 72 in total (12 pigs x 6 treatments)) were scanned on d 27 using DXA. The pigs were removed from feed and fasted for approximately 16 hr before scanning. Immediately before 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 BW. When sufficiently sedated the pigs were transferred to the DXA machine (Norland XR46 Densitometer Machine) (Suster *et al.* 2006). 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 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.

8.5 Statistical analysis

General analysis of variance was performed with the GENSTAT 17 program (VSN International Ltd, Hemel Hempstead, UK) to analyse the main effects of sex and diet on growth performance, carcass quality, body composition and physiological measures. Batch was used as a block in the analysis. A level of probability of less than 0.05 was used to determine statistical difference between the means. A level of probability of less than 0.1 but greater than 0.05 was determined to be a trend. Fisher's-protected least significant differences were used to determine differences among treatments.

9. Experiment 2 Results

9.1 Growth and carcass performance

The results for growth and carcass performance are given in Table 8. Immunocastrated males grew faster (P=0.005), ate more feed (P=0.046) and had a better feed conversion (P=0.032) compared to entire males from d 0-14. From d 15-28 there was a trend for IC males to grow slower (P=0.062), eat more feed (P=0.001) and have a poorer feed conversion ratio (P<0.001) compared to entire males. From d 0-28 there was no difference in daily gain (P=0.671), however, IC males ate more feed (P=0.001) and had a poorer feed conversion compared to entire males (P<0.001).

Pigs fed Albus 28 had a lower daily gain (P<0.001), lower feed intake (P<0.001) and a trend for a poorer feed conversion ratio (P=0.077) compared to pigs fed the control diet or Albus 14 from d 0-14. From d 15-28 pigs fed Albus 14 had a lower daily gain (P<0.001) and lower feed intake (P<0.001) compared to pigs fed Albus 28 which in turn had a lower daily gain and feed intake than those on the control diet (P<0.001). Pigs fed the Albus 14 diet had a poorer feed conversion (P=0.06) compared to the Albus 28 diet. There was no difference in feed conversion between those on the Albus 14 and control diet or between the Albus 28 and control diet. From d 0-28 pigs fed the Albus 14 or an Albus 28 diet had a lower daily gain (P<0.001) and a lower feed intake (P<0.001) compared to the control diet. There was trend for pigs fed the Albus 14 diet to have a poorer feed conversion (P=0.091) than those fed either the control or Albus 28 diet.

From d 15-28 IC males receiving the Albus 14 diet grew slower (P<0.001) than entire males receiving the Albus 14 diet. From d 0-28 (P=0.009) there was a sex by diet interaction in that IC males fed the control diet grew faster than entire males fed the control diet but there was no difference in growth rate between sexes for either Albus 14 or Albus 28. There was a sex by diet interaction for feed intake for d 15-28 (P<0.001) where IC males fed the control diet ate more feed than entire males fed the control diet, however, there was no difference between sexes in feed intake of the Albus 14 and Albus 28 diet. There was a sex by diet interaction from d 0-28 for feed take (P=0.009) where IC males fed Albus 28 had a lower feed intake than IC males fed Albus 14 but there was no difference in feed intake between Albus 14 and Albus 28 for entire males. There was a sex by diet interaction for the feed conversion ratio from d 15-28 (P=0.034) where IC males fed Albus 14 had a worse feed conversion ratio compared to those fed either Albus 14 or the control diet. There was no difference between diets for the entire males.

There was no effect of sex on carcass weight (P=0.415). There was a trend for IC males to have a lower dressing percentage than entire males (P=0.059). Immunocastrated males were approximately 1 mm fatter than entire males at the P2 site (P<0.001).

Pigs fed either Albus 14 or Albus 28 had a lower carcass weight (P<0.001) compared to those fed the control diet. Diet had no effect on dressing percentage (P>0.05). Pigs fed either Albus 14 or Albus 28 had a lower backfat than those on the control diet (P=0.047).

There was a sex by diet interaction (P=0.027) for carcass weight where IC males fed either Albus 14 or Albus 28 had a lower carcass weight compared to those on the control diet, however, there was no difference between diets for entire males. There was also a sex by diet interaction (P=0.028) for dressing percentage where IC males fed Albus 28 had a lower dressing percentage compared to the

other diets, however, there was no difference between diets for entire males. There was a sex by diet interaction (P=0.042) for backfat where IC males had a lower backfat when fed either Albus 14 or Albus 28 compared to the control diet, however, there was no difference between diets for entire males.

Table 8: Growth and carcass performance for entire male and immunocastrated male pigs fed three different diets from 72.3 to 101.1 kg LW (n=7).

	Entire male			Immunocastrated male			SED[a]	P-value		
	Control	Albus 28	Albus 14	Control	Albus 28	Albus 14		Sex	Diet	S×D
Daily gain (kg/day)										
d 0-14	1.11	0.861	1.04	1.18	0.950	1.14	0.050	0.005	<0.001	0.925
d 15-28	1.12	1.09	0.929	1.28	0.929	0.726	0.060	0.062	<0.001	<0.001
d 0-28	1.11	0.974	0.985	1.23	0.940	0.933	0.040	0.671	<0.001	0.009
Feed intake (kg/day)										
d 0-14	2.72	2.29	2.64	2.78	2.34	2.83	0.084	0.046	<0.001	0.420
d 15-28	3.05	2.72	2.49	3.86	2.74	2.49	0.134	0.001	<0.001	<0.001
d 0-28	2.88	2.51	2.57	3.32	2.54	2.67	0.094	0.001	<0.001	0.009
Feed conversion ratio										
d 0-14	2.47	2.69	2.55	2.37	2.47	2.49	0.097	0.032	0.077	0.486
d 15-28	2.73	2.52	2.68	3.01	2.97	3.51	0.148	<0.001	0.006	0.034
d 0-28	2.59	2.58	2.61	2.70	2.70	2.87	0.067	<0.001	0.091	0.247
CW (kg)[c]	67.7	65.9	65.3	69.8	62.8	64.4	1.28	0.415	<0.001	0.027
DP (%)[d]	65.4	65.6	65.6	65.7	64.2	65.2	0.421	0.059	0.111	0.028
P2 backfat (mm)[b]	9.28	8.90	9.07	11.1	9.66	9.49	0.391	<0.001	0.047	0.042

[a] SED for Sex × Diet

[b] Carcass weight used as a covariate.

[c] CW – carcass weight

[d] DP – dressing percentage

9.2 Body composition

The body composition results are given in Table 9. The percentage bone mineral content (BMC) was not affected by sex (P=0>0.05) or diet (P>0.05). There was a sex by diet interaction (P=0.001) in that IC males on the control diet had a lower BMC than those on the albus diet, however there was no difference between diets for the entire males. Immunocastrated males had a lower percentage lean mass (P<0.001) and a higher percentage fat deposition (P<0.001) compared to entire males. There was a trend for pigs on the Albus 14 and Albus 28 diets to have a higher lean deposition (P=0.055) and a lower fat deposition (P=0.056) compared to the pigs on the control diet. There were no interactions for lean deposition or fat deposition.

Table 9: Body composition for entire male and immunocastrated male pigs fed three different diets from 72.3 to 101.1 kg LW (n=12).

	Entire male			Immunocastrated male			SED[a]	P-value		
	Control	Albus 28	Albus 14	Control	Albus 28	Albus 14		Sex	Diet	S×D
% BMC[b]	1.88	1.75	1.82	1.70	1.86	1.79	0.053	0.282	0.913	0.001
% Lean	82.5	83.7	82.9	79.1	81.4	81.7	1.071	<0.001	0.055	0.334
% Fat	15.6	14.6	15.3	19.2	16.8	16.5	1.083	<0.001	0.056	0.288

[a] SED for Sex×Diet

[b] BMC – bone mineral content

10. Experiment 2 Discussion

The hypothesis that pigs immunised against GnRF which are fed a diet containing albus lupins, for either 14 or 28 days prior to slaughter, will have a reduced feed intake and growth rate compared to pigs immunised against GnRF receiving a standard finisher diet, was supported. The feed intake of IC males was 23% less for pigs on the Albus 28 diet and 20% less for the Albus 14 diet compared to the IC males receiving the control diet. The growth rate was 23% less for IC males on the Albus 28 diet and 24% less for receiving the Albus 14 diet compared to control. As outlined in Experiment 1 albus lupins are thought to decrease feed intake by delayed transit through the stomach and small intestine. This may then feedback through satiety signals (Dunshea *et al.* 2001).

In contrast to Experiment 1, the decrease in the level of feed intake and growth rate in IC males receiving the albus lupin diet was of an increased magnitude. In Experiment 1 the IC males on the 20% albus lupin diet had a similar daily gain, feed intake and feed conversion compared to entire males fed the control diet for d 15-28. However, for the same period of time in Experiment 2 the daily gain and feed intake of the IC males on the albus diets were lower than the entire males on the control diet and the feed conversion rate was also worse for the IC males. The standardised ileal digestible lysine levels of the diets were as expected but it appears there may have been increased acceptability issues with the albus low diet (diet received from d 15-28 for the IC males). It is suggested that further work examine titrating the inclusion level of albus lupins.

It should also be noted that the greater than expected restriction of feed intake (-25%) affected the daily gain of IC males to a greater extent than entire males. This was probably because the entire males have faster and leaner growth than IC males at a similar level of energy intake because they have a greater capacity for lean tissue growth (Campbell and Taverner, 1985).

The hypothesis that pigs fed albus lupins will have less fat compared to pigs receiving a standard finisher diet was supported. Pigs on both of the albus lupin diets had approximately 2.5% less body fat and 0.9 mm lower backfat compared to pigs fed the control diet. This concurs with findings from Quiniou *et al.* (2012) who when restricting feed intake to 2.5 or 2.75 kg/d, which equated to 15 and 22% lower feed intake than *ad libitum,* found that backfat thickness was reduced by between 0.6 and 1.0 mm in the restricted fed pigs compared to the *ad libitum* fed pigs. The reduction in fat composition and backfat for pigs fed the albus lupin diets was greater for IC males compared to entire males. This is likely because of the increased fat deposition associated with IC males two weeks after the second immunisation against GnRF as demonstrated In Experiment 1 and Moore *et al.* (unpub).

The hypothesis that pigs immunised against GnRF and fed a diet containing albus lupins for either 14 or 28 days prior to slaughter will have a similar backfat compared to entire males receiving a standard finisher diet was supported. This result concurs with Experiment 1 where IC male pigs fed albus lupins for 28 days had a similar backfat to entire males fed the control diet for 28 days.

The hypothesis that pigs immunised against GnRF and fed L. albus for 28 days prior to slaughter will have a lower overall daily feed intake but a similar fat composition compared to pigs immunised against GnRF and fed L. albus for 14 days prior to slaughter was also supported. Immunocastrated male pigs fed albus lupins for 28 days ate 5% less feed overall than IC males fed albus lupins for 14 days pre-slaughter, while their percentage fat composition was similar. However, when the feed intake was compared for the d 15-28 only, IC males that were fed albus lupins for this period only (Albus 14) had a 9% lower feed intake than those that had received albus lupins for the entire 28 days (Albus 28).

Therefore, the albus lupin diet would only need to be fed for the final two week period before slaughter to minimise fat deposition and the increase in backfat, provided the large adverse effect on growth rate can be alleviated.

11. Implications & Recommendations

A combination of calcium chloride (3%) and sodium tripolyphosphate (1.6%) can successfully be used to improve feed conversion by reducing feed intake, in this instance by 9%, with no effect on growth performance in the finishing period. In this experiment, it was not effective at reducing backfat in IC male pigs. The ability to manipulate voluntary feed intake may be useful to regulate seasonal impacts on finisher pig growth performance (Pluske *et al.* 2015).

In Experiment 2 the inclusion of albus lupins at 20% in the diets of IC male pigs was successful at reducing feed intake, body fat and backfat to similar levels of entire males. However, the growth rate of the IC male pigs was adversely impacted more than would be desirable. However, in Experiment 1 when albus lupins where included at 20% for the last 14 days pre-slaughter the feed intake, growth rate and backfat of IC males were similar to that of entire males. Due to the inconsistent results on the growth rate of IC males, further investigation on the effect of albus lupins in the diets of immunocastrated male pigs is warranted. It is suggested that the effect of albus lupins on growth performance and backfat be further investigated using titrated levels of albus lupins, for example, from 10% to 20% to determine an appropriate level to include in order to maximise the decrease in feed intake and fat deposition whilst minimising the effect on growth rate.

12. Intellectual Property

There is no intellectual property arising from this research.

13. Technical Summary

No additional technical information on methodologies, equipment design etc. was developed as part of these experiments.

14. Literature cited

Batorek, N., Skrlep, M., Prunier, A., Louveau, I., Noblet, J., Bonneau, M., & Candek-Potokar, M. 2012. Effect of feed restriction on hormones, performance, carcass traits, and meat quality in immunocastrated pigs. Journal of Animal Science 90, 4593-4603.

Bouton, P.E., Harris, P.V. & Shorthose, W.R. 1971. Effect of ultimate pH upon the water holding capacity and tenderness of meat. Journal of Food Science 36, 435-439.

Campbell, R. G., and M. R. Taverner. 1985. Effect of stain and sex on protein and energy metabolism in growing pigs. In: R. W. Moe, H. F. Tyrell and P. J. Reynolds, editors, Energy Metabolism of Farm Animals. European Association of Animal Production Publication no. 32. Rowman and Littlefield, NJ. p. 78-81.

Cronin, G.M., Dunshea, F.R., Butler K.R., McCauley I., Barnett, J.L., & Hemsworth, P.H., 2003. The effects of immuno- and surgical-castration on the behaviour and consequently growth of group-housed, male finisher pigs. Applied Animal Behaviour Science 81, 111–126. doi: 10.1016/S0168-1591(02)00256-3

Dunshea, F.R. 2009. Castration in the swine industry: physical versus immunological. Pfizer International Swine Symposium – Proc. Ottawa, Canada: 26-32.

Dunshea, F.R., Calantoni, C., Howard, K., McCauley, I., & Jackson P. 2001a. Vaccination of boars with a GnRF vaccine (Improvac) eliminates boar taint and increases growth performance. Journal of Animal Science 79, 2524–2535.

Dunshea, F.R., Gannon, N.J., van Barneveld, R.J., Mullan, B.P., Campbell, R.G. & King, R.H. 2001b, Dietary lupins (*Lupinus angustifolius* and *Lupinus albus*) can increase digesta retention in the gastrointestinal tract of pigs. Australian Journal of Agricultural Research 52, 593-602.

Dunshea, F.R., Rikard-Bell, C., Curtis, M.A., Edwards, A.C., Gannon, N.J., Henman, D.J., Mullan, B.P., & van Barneveld, R.J. 2005. A step-up ractopamine (Paylean) program increases lean tissue in all sexes and decreases fat tissue in boars and immunocastrates. In "Manipulating Pig Production X" Australasian Pig Science Association, ed. J.E. Paterson, Werribee, 152.

Dunshea, F.R., Cronin, G.M., Barnett, J.L., Hemsworth, P.H., Hennessy, D.P., Campbell, R.G., Luxford, B., Smits, R.J., Tilbrook, A.J., King, R.H., & McCauley, I. 2011. Immunisation against gonadotrophin-releasing hormone (GnRH) increases growth and reduces variability in group-housed boars. Animal Production Science 51, 695-701.

Dunshea FR, Allison JRD, Bertram M, Boler DD, Brossard L, Campbell R, Crane JP, Hennessy DP, Huber L, de Lange C, Ferguson N, Matzat P, McKeith F, Moraes PJU, Mullan BP, Noblet J, Quiniou N, & Tokach M. 2013. The effect of immunisation against GnRF on nutrient requirements of male pigs: A review. Animal 7, 1769-1768.

Kim, J.C., Mullan, B.P., Nicholls, R.R., & Pluske, J.R. 2011. Effect of Australian sweet lupin (*Lupinus angustifolius* L.) inclusion levels and enzyme supplementation of the performance, carcass composition and meat quality of grower/finisher pigs. Animal Production Science 51, 37-43.

King, R.H., 1981. Lupin-seed meal (*Lupinus albus* cv. Hamburg) as a source of protein for growing pigs. Animal Feed Science and Technology 6, 285-296.

Lanferdini, E., Lovatto, P.A., Melchior, R., Orlando, U.A.D., Ceccantini, M., & Poleze, E. 2013. Feeding surgically castrated, entire male and immunocastrated pigs with different levels of amino acids and energy at constant protein to energy ratio with or without ractopamine. Livestock Science 151, 246-251.

Lealiifano AK, Pluske JR, Nicholls RR, Dunshea FR, Campbell RG, Hennessy DP, Miller DW, & Mullan BP. 2011. Reducing the length of time between harvest and the secondary GnRF immunization improves growth performance and clears boar taint compounds in male finishing pigs. Journal of Animal Science 89, 2782-2792.

Main, R. G., S. S. Dritz, M. D. Tokach, R. D. Goodband and J. L. Nelssen. 2008. Determining an optimum lysine:calorie ratio for barrows and gilts in a commercial finishing facility. Journal of Animal Science 86, 2190-2207.

McCauley, I., Watt, M., Suster, D., Kerton, D.J., Oliver, W.T., Harrell, R.J., & Dunshea, F.R., 2003. A GnRF vaccine (Improvac) and porcine somatotropin (Reporcin) have synergistic effects upon growth performance in both boars and gilts. Australian Journal of Agricultural Research 54, 11-20.

Moore, K.L., Dunshea, F.R., Mullan, B.P., Hennessy, D.P. & D'Souza, D.N. 2009. Ractopamine supplementation increases lean deposition in entire and immunocastrated male pigs. Animal Production Science 49, 1113-1119.

Moore, K. L., Mullan, B.P., Kim, J.C. & Dunshea, F.R. 2016. Standardized ileal digestible lysine requirements of male pigs immunized against gonadotrophin releasing factor. Journal of Animal Science, accepted doi:10.2527/jas2015-9622.

Oliver, W.T., McCauley, I., Harell, R.J., Suster, D., Kerton D.J., & Dunshea, F.R. 2003. A gonadotropin-releasing factor vaccine (Improvac) and porcine somatotropin have synergistic and additive effects on growth performance in group-housed boars and gilts. Journal of Animal Science 81, 1959–1966.

Ostrowska, E., Muralitharan, M., Cross, R.F., Bauman, D.E., & Dunshea, F.R. 1999. Dietary conjugated linoleic acids increase lean tissue and decrease fat deposition in growing pigs. Journal of Nutrition, 129, 2037-2042.

Pluske, J.R., Black, J.L., Kim, J.C., & Dunshea, F.R. 2015. Suppressing the feed intake of finisher pigs: a preliminary study. Animal Production Science 55, 1546. doi.org/10.107/ANv55n12Ab063

Quiniou, N., Monziols, M., Colin, F., Goues, T. & Courboulay, V. 2012. Effect of feed restriction on the performance and behaviour of pigs immunologically castrated with Improvac®. Animal 6(9), 1420-1426. doi:10.1017/S1751731112000444.

Rasmussen, A.J., & Andersson, M. 1996. New methods for determination of drip loss in pork muscles. In Meat for the Consuemr, *42nd International Congress of Meat Science and Technology* (pp. 286-287). Norway: Matforsk, Lillehammer.

Ramaekers, P.J.L., Swinkels, J.W.G.M., Huiskes, J.H., Verstegen, M.W.A., Den Hartog, L.A. & Van der Peet-Schwering, C.M.C. 1996. Performance and carcass traints of individual pigs housed in groups as affected by ad libitum and restricted feeding. Livestock Production Science 47, 43-50.

Rikard-Bell, C., Curtis, M.A., van Barneveld, R.J., Mullan, B.P., Edwards, A.C., Gannon, N.J., Henman, D.J., Hughes, P.E., & Dunshea, F.R. 2009. Ractopamine hydrochloride improves growth performance and carcass composition in immunocastrated boars, intact boars, and gilts. Journal of Animal Science 87, 3536-3543.

Soine, T.O. & Wilson, C.O. 1967. Roger's Inorganic Pharmaceutical Chemistry (8th Ed.). Lea and Febiger, Philadelphia, PA.

Suster, D., Leury, B.J., Kerton, D.J., Borg, M.R., Butler, K.L. & Dunshea, F.R. 2006. Longitudinal DXA measurements demonstrate lifetime differences in lean and fat tissue deposition between boars and barrows under individual and group-penned systems. Australian Journal of Agricultural Research 57, 1009-1015.

Van Nevel, C., Seynaeve, M., Van De Voorde, G., De Smet, S., Van Driessche, E. & de Wilde, R. 2000. Effects of increasing amounts of Lupinus albus seeds without or with whole egg powder in the diet of growing pigs on performance. Animal Feed Science and Technology 83, 89-101.

Yen, J.T., Pond, W.G., & Prior, R.L. 1981. Calcium chloride as a regulator of feed intake and weight gain in pigs. Journal of Animal Science 52, 778-782.

Zeng, X.Y., Turkstra, J.A., Jongbloed, A.W., van Diepen, J.Th.M., Meloen, R.H., Oonk, H.B., Guo, D.Z., van de Wiel, D.F.M. 2002. Performance and hormone levels of immunocastrated, surgically castrated and intact male pigs fed ad libitum high- and low- energy diets. Livestock Production Science 77, 1-11.

15. Publications Arising

There are no publications arising from this project.