DIETARY STRATEGIES TO ALLEVIATE THE IMPACT OF SEASONAL INFERTILITY WITHIN GILT POOLS
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Executive Summary

It is common during late summer and early autumn for domestic sows to experience a depression in fertility (seasonal infertility), which is frequently manifested as a reduced proportion of gilts reaching puberty and a decrease in litter size. Alterations in day length, mediated by changing levels of melatonin secretion, are ultimately responsible for seasonal infertility. Melatonin production is reduced during exposure to long photoperiods (i.e. during the summer months), essentially increasing the negative feedback effects of oestrogen on the GnRH pulse generator, thus suppressing episodic LH release. It is hypothesized that this suppressed pattern of LH release will decrease the size and maturity of the antral follicle pool. As a result, puberty attainment in response to boar contact will be delayed, and the number of follicles selected for ovulation and the quality of the oocytes shed, and thus embryo survival, will be decreased. It is, therefore, suggested that feeding diets during the rearing and pre-mating period which reduce the negative feedback effects of oestrogen during the summer months will increase LH pulse frequency, thus improving the timing of the pubertal response and increasing litter size of summer/autumn mated gilts. Previous studies indicate that clearance rate of oestrogen is increased when gilts are fed a high 'fibre' diet immediately prior to mating, resulting in increased ovulation rate and oocyte developmental competence.

The current project consisted of two studies designed to determine whether feeding a diet rich in either non-starch polysaccharides (whole lupins) or non-fermentable fibre (wheat bran) during the gilt rearing phase and prior to mating would; i) decrease circulating oestradiol concentrations, increase GnRH release and promote maturation of the follicle-oocyte complex; and ii) improve puberty attainment and litter size during summer/autumn. Study one was conducted in the summer of 2009 / 2010, and using 54 Large White / Landrace cross terminal line gilts, demonstrated that including lupin fibre (35%) in pre-mating diets improved the proportion of oocytes able to complete meiosis in vitro by 17% compared to standard fed gilts. However, the addition of wheat bran to the diet exerted neither a positive or negative effect on oocyte development in vitro compared to standard fed gilts. As a result of this, study two (conducted in the summer of 2010/2011) determined the effect of lupin fibre (35%) in pre-mating diets on puberty attainment and potential litter size on day 30 of gestation. The results of study two demonstrate no effect of lupin fibre on the timing of the pubertal response to boar contact or ovulation rate. However, gilts receiving the lupin based diet prior to mating possessed 2 more embryos than their standard fed counterparts (P = 0.054;13.8 versus 11.8), resulting in a 16% improvement in embryo survival (0.92 versus 0.76). Together, the current data demonstrate a positive effect of adding lupin fibre to diets fed prior to mating (at least during summer) on oocyte developmental competence and potential litter size. However, future studies are required to 1) validate this effect on a commercial scale; 2) determine whether the observed improvements in embryo survival translate into more piglets being born; and, 3) identify additional fibre sources (preferably available throughout Australia) which improve litter size in female pigs.

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1. Introduction

It is common during late summer and early autumn for domestic sows to experience a depression in fertility (seasonal infertility), which is frequently manifested as a reduced proportion of gilts reaching puberty and a decrease in litter size. Alterations in day length, mediated by changing levels of melatonin secretion, are ultimately responsible for seasonal infertility (Tast et al., 2001; Peltoniemi et al., 2005). Melatonin suppresses the negative feedback effects of oestrogen on the GnRH pulse generator, thus exerting a stimulatory effect on episodic release of GnRH and luteinising hormone (LH). Melatonin production is reduced during exposure to long photoperiods (i.e. during the summer months), essentially increasing the negative feedback effects of oestrogen on the GnRH pulse generator, thus suppressing episodic LH release. This suppressed pattern of LH release decreases the size and maturity of the antral follicle pool, thus delaying puberty attainment in response to boar contact and decreasing the number of follicles selected for ovulation. Suppressed LH release will also result in a higher proportion of the oocytes shed during summer /early autumn being developmentally 'incompetent' and incapable of completing the early stages of embryo development, surviving implantation and become piglets (Hunter et al., 2004). It takes approximately 104 days for the ovarian follicle to reach the ovulatory stage, and throughout this entire period the follicle-oocyte complex is profoundly sensitive to changes in the metabolic and endocrine environment (Hunter, 2004; van Wettere and Hughes, 2007). As a result, the impaired LH release that occurs during summer will negatively impact the follicles and oocytes selected and ovulated during both the summer and early-autumn period.

It is, therefore, suggested that feeding diets during the rearing and pre-mating period which reduce the negative feedback effects of oestrogen during the summer months will increase LH pulse frequency, thus improving the timing of the pubertal response and increasing litter size of summer/autumn mated gilts. Clearance rate of oestrogen is increased when gilts are fed a high 'fibre' diet immediately prior to mating, resulting in increased ovulation rate and oocyte developmental competence (Ferguson et al., 2007). One-third to one-half of circulating oestrogens are incorporated into the bile, with 80% of these oestrogens reabsorbed through the gut wall; however, binding of oestrogen to dietary fibre in the gut will reduce the amount of oestrogen that is reabsorbed, thereby increasing the amount of oestrogen excreted in the faeces and lowering circulating oestrogen concentrations (Arts et al., 1991). It has been demonstrated *in vitro* that oestrogens bind with the highest affinity to specific fibre sources, with wheat bran identified at an excellent binder of oestrogen. Further, feeding fibre sources that are rich in fermentable non-starch polysaccharides (NSPs) also increases hindgut fermentation, increasing the availability of volatile fatty acids (VFAs) as an energy source, and stabilising interprandial blood glucose and insulin levels (de Leeuw et al., 2002; Johnston et al., 2003), thus stimulating increased frequency of LH pulse release and ovarian follicle growth (Cosgrove and Foxcroft, 1996). Higher levels of VFAs can also promote GnRH release (Boukhliq and Marting, 1997). The objective

of the current project was to determine whether feeding a diet rich in either non-starch polysaccharides (whole lupins) or non-fermentable fibre (wheat bran) during the gilt rearing phase and prior to mating would; i) decrease circulating oestradiol concentrations, increase GnRH release and promote maturation of the follicle-oocyte complex; and ii) improve puberty attainment and litter size during summer/autumn.

2. Methodology

Experiment One: Effect of feeding high fibre diets to gilts during summer on endocrine profiles and oocyte developmental competence

The study was run in three blocks during summer / early autumn of 2009/2010: block one ran from December to February, block two ran from January to March and block three ran from February to April.

Experimental animals, housing and management

At 133 days of age, fifty-four Large White cross terminal line pre-pubertal gilts were selected. Gilts were weighed at 154 days of age, stratified according to weight and allocated to receive 3kg / day of either a standard female finisher diet (Control) supplying 13.25 MJ digestible energy (DE), 15.3% protein, and 3.9% fibre, a Lupin based high fibre diet (Lupin) supplying 13.22 MJ DE, 18.6% protein and 11.8% fibre, or a bran based high fibre diet (Bran) supplying 13.20 MJ DE, 15.4% protein and 6.5% fibre (Table 1). Gilts were group-housed in their treatment groups for the duration of the experiment except during catheterisation and frequent blood sampling, when they were housed individually.

Puberty stimulation and oestrus detection

From selection at 133 days until the commencement of puberty stimulation, gilts had no exposure to male pigs. From 154 days of age through to puberty, gilts were checked daily in their pens, without boar contact, for vulval swelling and reddening as well as signs of behavioural oestrus. At 175 days of age, puberty was stimulated using a combination of PG600 (400IU Pregnant Mare Serum Gonadotrophin and 200IU human Chorionic Gonadotrophin; Intervet, Australia) and boar contact (Bartlett *et al.*, 2009). Specifically, gilts were restrained and injected intramuscularly with PG600 behind the ear. Following injection, each treatment group, as penned, were taken to a detection mating area (DMA), where they received 20 minutes of supervised full contact with a mature boar. Gilts received daily boar exposure until the attainment of puberty, and then from approximately day 15 to day 19 of the subsequent oestrous cycle. Boar exposure commenced at 09:00 hours, with oestrus defined as the exhibition of a standing reflex in response to the manual application of pressure to the gilt's back (the "backpressure" test). The attainment of puberty was defined as the first sign of a standing reflex.

Table 1. Formulation of the experimental diets.

	Treatment diets		
	Control	Bran	Lupin
Composition of base diets (%)			
Wheat	44.43	36.13	24.33
Barley	40.33	1	27.67
Millmix	5		
Bran		50	
Lupins			20
Lupin hulls			15
Canola 35. Exp. Mill	2		
Meatmeal	3.83	5.33	7.5
Bloodmeal	0.67	0.33	
Tallow-mixer	0.67	5	4.17
Salt	0.2	0.2	0.2
Limestone – MB	1.33	1.07	0.6
Alimet		0.03	0.06
Threonine	0.12	0.03	
Lysine Sulphate	0.44	0.45	0.12
Choline Chloride	0.04	0.06	0.01
Mycosorb	0.1	0.1	0.1
L/A – 1007 Sow Premix + Bioplex	0.28	0.28	0.28
Biofos – MDCP	0.67		
Daily nutrient allowance			
Digestible energy (MJ)	13.25	13.20	13.22
Protein (%)	15.28	15.39	18.65
Fibre (%)	3.86	6.51	11.77
Fat (%)	3.16	8.28	7.33

Animal measurements: liveweight and P2 backfat

Gilts were weighed and P2 backfat measured at 133, 154 and 175 days of age, and on day 19 of the first oestrous cycle. P2 backfat depth was measured over the last rib, 65 mm from the vertebrae, using a 5 MHz linear probe (Aquila Vet, Pie Medical Equipment).

Animal measurements: blood collection

At 175 days of age, prior to PG600 injection, a preprandial blood sample was taken by jugular venipuncture into a 9 mL EDTA-coated collection tube (Vacuette®, Griener Labortechnik, Austria). Blood samples were maintained on ice and processed within an hour of collection. Blood samples were centrifuged for fifteen minutes at 3000 rpm. Plasma was stored at -20 °C.

On day 15 of the first oestrous cycle, nine gilts from each treatment group were selected based on body weight to be fitted non-surgically with an indwelling jugular vein cannula via an ear vein. The method was modified from Virolainen *et al*. (2005). Blood samples were taken twice daily (8am and 8pm) on days 16 and 17 of the oestrous cycle. On day 18, a blood sample was taken at 8am followed by a 10 hour pulse bleeding session with blood sampling every 15 minutes. Samples were collected into a heparin (250 IU) primed 3 ml syringe and the catheter was then flushed with 100 IU heparin. Blood samples were centrifuged at 2200 rpm for 10 minutes and the plasma stored at -20°C.

Ovary and oocyte recovery

Gilts were slaughtered on day 19 of the oestrous cycle at a local abattoir, and the reproductive tract of each animal was recovered. Ovaries were separated from the rest of the reproductive tract, placed in individual 50 mL falcon tubes containing phosphate buffered saline (PBS) and maintained at 30°C during transport to the laboratory.

Ovaries were maintained at 30°C during processing at the laboratory. Ovaries from each gilt were weighed. The 15 largest follicles across both ovaries were measured and follicular contents were aspirated using an 18 G needle and a vacuum pump (Cook Australia, Queensland, Australia) set at 33 mm Hg. Oocytes were recovered from the 15 largest follicles on the assumption that the largest follicles present in the late follicular phase represent the presumptive ovulatory pool (as described by Foxcroft *et al.,* 1987 and Ferguson *et al.,* 2003). The selection of 15 follicles was based on a previous study involving the same genotype and puberty stimulation protocol (Bartlett *et al.,* 2009) in which ovulation rate was recorded for gilts mated at their second oestrus. Following aspiration, the aspirate was searched for cumulus-oocyte complexes (COCs) under a dissecting microscope. For each gilt's ovaries, surface follicles were characterised according to three size categories; 1 – 2.9 mm, 3 – 4.9 mm or > 5 mm in diameter. The number of follicles in each size category were counted.

Oocyte maturation, staining and assessment of meiotic progression

COCs were washed three times in aspiration media and once in maturation medium. The COC's were placed in wells containing 600 µL of maturation medium under 300 µL of equilibrated mineral oil (Sigma) and incubated for 40-44 hours at 37.8°C under humidified 5% CO_2 in air.

After maturation, COCs were denuded of cumulus cells using hyaluronidase (Sigma) and a fine bore pipette. The oocytes were stained with Hoescht 33342 stain and assessed for nuclear maturation stage. Oocytes were identified under ultraviolet light and classified as described by Ye *et al*. (2002): germinal vesicle (GV), germinal vesicle breakdown (GVBD), metaphase I (MI), anaphase-telophase (A-T) or metaphase II (MII).

Hormone analysis

All plasma analyses were conducted by the Adelaide Research Assay and Services Facility, University of Adelaide.

Luteinising hormone (LH)

All plasma samples were assayed for LH concentration using reagents from the National Hormone and Peptide Program, USA. One hundred uL samples were assayed in duplicate. The LH standard was highly purified Porcine LH (AFP.11043B) and the lowest detectable concentration was 0.39 ng mL^{-1}.

Oestradiol

Oestradiol concentrations were determined by radioimmunoassay using an Ultra Sensitive Estradiol RIA kit (Beckman Coulter/DSL; Kit #DSL4800). Oestradiol was measured in samples collected at 175 days of age, and on days 16 and 17 of the oestrous cycle as well as the first and last sample collected during the ten hour pulse bleeds on day 18 of the oestrous cycle. Two hundred uL in single aliquots was assayed with the lowest detectable concentration of 2.7 pg mL^{-1}.

Statistical analysis

Values in the text are expressed as Mean ± SEM. LH profiles on day 18 were visually appraised and deemed to be a pulse if (i) the peak occurred within 3 samples of the previous nadir, (ii) there were at least two samples between the peak and the subsequent nadir and (iii) the peak was greater than 0.3 ng mL^{-1} above basal concentration. This classification system was based on McLeod and Craigon (1985), and adapted based on van den Brand *et al.* (2000) and Langendijk *et al.* (2007). The distribution of surface antral follicles within the described size categories is expressed as the proportion of follicles per size class relative to the total number of surface antral follicles greater than 1 mm in diameter. All proportions were transformed by arcsin square root transformation prior to analysis. A general analysis of variance model, with block built in, was used to study the effects of diet on all variables measured. Differences between treatments were examined using least significant difference, with differences considered significant when $P < 0.05$.

Experiment Two: Effect of feeding high fibre diets to gilts during summer on puberty attainment and potential litter size

The study was run in two blocks during summer / early autumn of 2010/2011: block one ran from December to March, block two ran from January to March and block three ran from February to May.

At 148 days of age, sixty-four Large White cross terminal line pre-pubertal gilts were selected. Gilts were weighed at 160 days of age, stratified according to weight and allocated to receive 3kg / day of either a standard female finisher diet

(Control) supplying 13.25 MJ digestible energy (DE), 15.3% protein, and 3.9% fibre or a Lupin based high fibre diet (Lupin) supplying 13.22 MJ DE, 18.6% protein and 11.8% fibre (Table 1). Gilts were group-housed in their treatment groups until their second oestrus, at which point they were housed individually and received 2.2 kg per day of the Control diet up until slaughter.

Puberty stimulation and oestrus detection

From selection at 148 days until the commencement of puberty stimulation, gilts had no exposure to male pigs. From 154 days of age through to puberty, gilts were checked daily in their pens, without boar contact, for vulval swelling and reddening as well as signs of behavioural oestrus. At 169 days of age, puberty was stimulated using daily physical contact with a mature boar in a detection mating area (DMA). Specifically, gilts were taken to a DMA, where they received 20 minutes of supervised full contact with a mature boar. Gilts received boar exposure until first detection of their second oestrus. Boar exposure commenced at 09:00 hours, with oestrus defined as the exhibition of a standing reflex in response to the manual application of pressure to the gilt's back (the "backpressure" test). The attainment of puberty was defined as the first sign of a standing reflex.

Animal measurements: liveweight and P2 backfat

Gilts were weighed at 154 and 169 days of age, at puberty, second oestrus and slaughter. P2 backfat depth was measured at 154 and 169 days of age, with measurements taken over the last rib, 65 mm from the vertebrae, using a 5 MHz linear probe (Aquila Vet, Pie Medical Equipment).

Reproductive measures

At their second oestrus, gilts were artificially inseminated twice (24 hours apart). Gilts were slaughtered on day 28.7 ± 0.19 post-mating, where day 0 equals the first 24 hours after first detection of oestrus. Post-slaughter, the reproductive tracts were collected and the following measurements taken; number of corpora lutea (CL), individual CL weight, number of viable embryos, embryo crown-rump length, embryo weight, wet placental weight, embryo:placental weight, and weight of the empty uterine horns.

3. Outcomes

Experiment One: Effect of feeding high fibre diets to gilts during summer on endocrine profiles and oocyte developmental competence

Of the 54 gilts allocated to this experiment, a total of 12 gilts were removed from the analyses. In the Control, Bran and Lupin treatments, respectively, 1, 2, and 3 gilts failed to express oestrus within 7 days of the start of puberty and were

removed from the trial. Three gilts had ovulated prior to ovary collection, (2 Controls and 1 Lupin treated) and the ovaries of 2 gilts from the bran fed group and 1 gilt from the lupin fed group were damaged during collection. Consequently, the data presented below relate to 15, 14 and 13 gilts from the Control, Bran and Lupin treatments respectively.

Gilt liveweight and body composition

At 154 days of age, prior to the dietary treatments, gilt liveweight and P2 backfat was similar for all treatment groups (Table 2). There were no treatment effects on liveweight at 175 days of age or on liveweight change from 154 to 175 days of age (Table 2; $P > 0.05$). However, compared to gilts on the Control diet, gilts on Lupin and Bran diet had less P2 back fat ($P < 0.05$) at 175 days of age. At slaughter, Control gilts were similar in weight to Bran and Lupin gilts; however, Lupin fed gilts were significantly heavier than Bran fed gilts ($P < 0.05$). P2 backfat at slaughter was significantly higher ($P < 0.05$) for Control compared to both Lupin and Bran fed gilts.

Reproductive measures

Puberty attainment

Mean days to puberty were similar ($P = 0.55$) in the Control, Bran and Lupin treatment groups: 3.73 ± 0.63, 3.94 ± 0.65 and 5.52 ± 0.68 days respectively.

Ovarian and uterine morphology

Diet did not significantly affect uterine or ovarian weight ($P > 0.05$, Table 3). There was no effect of diet on the mean number of surface antral follicles greater than 1 mm in diameter (Table 3). Similarly, the proportion of follicles measuring 1 – 2.9 mm, 3 – 4.9 mm, or > 5 mm was unaffected by diet (Table 3).

Table 2. Liveweight (LW), P2 backfat (P2) and rate of liveweight gain (LWG) for 154 and 175 day-old Control, Lupin and Bran fed gilts.

	Treatment diet		
	Control	Bran	Lupin
LW at 154 days of age (kg)	93.8 ± 1.0	93.3 ± 1.0	94.8 ± 1.0
P2 backfat at 154 days of age (mm)	12.8 ± 0.7	11.4 ± 0.7	10.4 ± 0.1
LW at 175 days of age (kg)	112.7±1.4	109.1 ± 1.4	113.9 ± 1.49
P2 backfat at 175 days of age (mm)	14.3 ± 0.7^{a}	11.9 ± 0.7^{b}	11.6 ± 0.7^{b}
LW at slaughter (kg)	129.7 ± 1.5^{ab}	126.6 ± 1.5^{a}	133.6 ±1.6^{b}
P2 backfat at slaughter (mm)	15.3 ± 0.6^{a}	12.3 ± 0.6^{b}	12.4 ± 0.6^{b}

LWG: d 154 to slaughter (kg/day)	0.82 ± 0.03	0.77 ± 0.03	0.87 ± 0.03
P2 gain: d 154 slaughter (mm/day)	0.06 ± 0.01	0.02 ± 0.01	0.05 ± 0.02

Means with superscript letters (a, b and c) in a row are significantly different.

Table 3. Uterine and ovarian weight, and distribution of surface follicles within three size categories (1 – 2.9 mm, 3 – 4.9 mm and >5 mm) on day 19 of the oestrous cycle of Control, Bran and Lupin gilts

	Treatment diet		
	Control (n=15)	Bran (n=14)	Lupin (n=13)
Uterine weight (g)	786.2 ± 38.3	758.2 ± 39.0	903.8 ± 66.0
Total ovarian weight (g)	14.1 ± 0.7	14.2 ± 0.6	14.0 ± 0.5
Total no. surface follicles > 1 mm	44.6 ± 8.4	57.8 ± 10.6	51.5 ± 9.3
Proportion of 1 – 2.9 mm follicles	0.59 ± 0.07	0.66 ± 0.07	0.66 ± 0.07
Proportion of 3 – 4.9 mm follicles	0.15 ± 0.03	0.16 ± 0.03	0.18 ± 0.04
Proportion of > 5 mm follicles	0.26 ± 0.06	0.18 ± 0.06	0.16 ± 0.04

Presumptive ovulatory follicles

Table 4 shows the measurements from the presumptive pre-ovulatory pool of the 15 largest follicles. There were no significant differences in follicle sizes or in the size range across the dietary treatments. All three treatments had an average follicle size for the 15 largest follicles of approximately 7 mm and the smallest of the 15 follicles was greater than 5 mm in all dietary groups.

Oocyte developmental competence

Of the three dietary treatments, a significantly higher percentage of oocytes collected from Lupin fed gilts reached MII *in vitro* compared to those collected from Control and Bran fed gilts (Table 5). Control fed gilts had the highest percentage of oocytes at GV (10.22 ± 2.32) compared to Bran (6.34 ± 2.4) and Lupin (1.29 ± 2.5) fed-gilts ($P < 0.05$). The percentage of oocytes at GVBD was significantly higher ($P < 0.05$) for Bran and Control fed gilts compared to Lupin fed gilts (Table 5). The percentage of oocytes at MI and A-T was similar for all three treatments. Although 15 follicles from each animal were aspirated, not all oocytes were located in the aspirate. Numbers assessed for each treatment group are indicated in Table 5.

Table 4. Mean, minimum, maximum and variation in diameter of the 15 largest follicles (presumptive ovulatory pool) present on both ovaries on day 19 of the oestrous cycle of Control, Bran and Lupin gilts.

	Treatment diet		
Follicle diameter	Control (n = 15)	Bran (n = 14)	Lupin (n = 13)

Mean (mm)	7.5 ± 0.3	7.0 ± 0.3	7.1 ± 0.3
Minimum (mm)	6.1 ± 0.3	5.6 ± 0.3	5.8 ± 0.3
Maximum (mm)	8.7 ± 0.4	8.2 ± 0.4	8.4 ± 0.4
Variation (mm)	2.6 ± 0.7	2.5 ± 1.1	2.6 ± 0.5

Reproductive hormones: LH and oestradiol

There was no effect of dietary treatments on either LH or oestradiol at any time point where a blood sample was taken. There was no significant difference in LH at 175 days of age for Control (1.28 ± 22 ng mL^{-1}), Bran (1.05 ± 0.23 ng mL^{-1}) and Lupin (0.85 ± 0.23 ng mL^{-1}). There was also no significant difference in oestradiol at 175 days of age for Control (4.08 ± 0.28 pg mL^{-1}), Bran (3.52 ± 0.29 pg mL^{-1}) or Lupin (3.80 ± 0.3 pg mL^{-1}).

Oestradiol and LH concentrations on days 16 and 17 of the oestrous cycle were unaffected by dietary treatment ($P > 0.05$; Table 5). There was no effect of diet on the number of LH pulses, or pulse characteristics, during the 10 hour sampling period on day 18 of the oestrous cycle (Table 6). Similarly, basal and mean LH were unaffected by diet (Table 7).

Table 5. Percentage of pre-ovulatory oocytes at the different stages of nuclear maturation from gilts fed either the Control, Bran or Lupin diets.

	Treatment diet		
	Control (n = 15)	Bran (n = 14)	Lupin (n = 13)
Stage of nuclear maturation			
Germinal vesicle (%)	10.2± 2.3^{a}	6.3 ± 2.0^{b}	1.3 ± 2.5^{b}
Germinal vesicle breakdown (%)	8.7 ± 3.0^{a}	17.3 ± 3.1^{b}	2.5 ± 3.2^{a}
Metaphase I (%)	10.3 ± 3.0^{a}	7.2 ± 3.2^{a}	7.4 ± 3.3^{a}
Anaphase – Telophase (%)	0.5 ± 1.0^{a}	3.2 ± 1.0^{a}	0.00 ± 1.1^{a}
Metaphase II (%)	71.6 ± 4.6^{a}	65.4 ± 4.8^{a}	88.9 ± 4.8^{b}

Means with superscript letters (a, b and c) in a row are significantly different.

Table 6. LH and oestradiol concentrations on days 16, 17 and 18 of the first oestrous cycle.

	Treatments diets		
	Control	Bran	Lupin
LH			
Day 16 of the oestrous cycle (ng mL^{-1})	1.46 ± 0.20	1.10 ± 0.22	1.27 ± 0.21
Day 17 of the oestrous cycle (ng mL^{-1})	1.38 ± 0.20	1.01 ± 0.21	1.34 ± 0.20
Day 18 of the oestrous cycle (ng mL^{-1})	1.37 ± 0.12	0.96 ± 0.13	1.12 ± 0.13
Oestradiol			
Day 16 of the oestrous cycle (pg mL^{-1})	3.92 ± 0.41	4.02 ± 0.44	4.44 ± 0.43
Day 17 of the oestrous cycle (pg mL^{-1})	4.75 ± 0.47	4.21 ± 0.49	4.31 ± 0.48
Day 18 of the oestrous cycle (pg mL^{-1})	4.78 ± 0.72	4.83 ± 0.76	4.89 ± 0.74

Table 7. Characteristics of pulsatile luteinising hormone release over 10 hours on day 18 of the first oestrous cycle.

	Treatment diets		
	Control	Bran	Lupin
No. Pulses per 10 h	3.85 ± 0.46	4.06 ± 0.49	4.61 ± 0.48
Mean LH pulse amplitude (ng mL^{-1})	0.62 ± 0.05	0.52 ± 0.09	0.55 ± 0.04
Average pulse area (ng mL^{-1})	4.02 ± 0.47	3.55 ± 0.33	3.84 ± 0.66
Total pulse area (ng mL^{-1})	14.86 ± 2.49	13.84 ± 2.82	18.34 ± 4.50
Mean LH concentration (ng mL^{-1})	2.27 ± 0.30	1.88 ± 0.32	2.40 ± 0.31
Basal LH concentration (ng mL^{-1})	0.86 ± 0.11	0.64 ± 0.12	0.89 ± 0.12

Experiment Two: Effect of feeding high fibre diets to gilts during summer on puberty attainment and potential litter size

Of the 64 gilts allocated to this experiment, a total of 2 gilts were removed from the analyses. One gilt was removed from each of the Control and Lupin treatments, due to structural issues. Consequently, unless otherwise stated the data presented below relate to 31 gilts per treatment.

Gilt liveweight and body composition

There was no effect of dietary treatment on gilt LW or P2 at any stage during the experimental period (Table 8).

Table 8. Liveweight (LW) and P2 backfat (P2) for Control and Lupin fed gilts

	Treatment diet	
	Control	Lupin
LW at 160 days of age (kg)	106.7 ± 1.0	107.7 ± 1.0
P2 backfat at 160 days of age (mm)	11.5 ± 0.3	11.4 ± 0.3
LW at 169 days of age_1 (kg)	111.4 ± 1.0	112.3 ± 1.0
P2 backfat at 169 days of age_1 (mm)	11.7 ± 0.3	11.4 ± 0.3
LW at first oestrus (kg)	119.4 ± 1.3	117.1 ±1.2
LW at second oestrus (kg)	133.7 ± 1.6	134.3 ± 1.5

Reproductive measures

Puberty attainment

Mean days to puberty were similar (P = 0.58) in the Control and Lupin treatment groups: 10.8 ± 1.20 and 9.8 ± 1.25 days respectively. The proportion of gilts

expressing oestrus within 35 days of the start of treatment was similar for the Control (0.84) and Lupin treatments (0.87).

Ovarian and uterine morphology

Three Control gilts and 2 Lupin gilts failed to express a second oestrus within 30 days of their pubertal oestrus. In addition, 2 Control and 2 Lupin gilts were not pregnant at time of slaughter. Consequently, the data presented in this section relates to 24 and 25 gilts for the Control and Lupin diet, respectively.

There was no effect of treatment (P = 0.54) on the total number of corpora lutea (CL) or luteal weight (Table 9). However, both the number of embryos tended (P = 0.054) to be higher, and the number of embryos expressed as a proportion of CL (embryo survival) was significantly (P = 0.036) higher for Lupin compared to Control gilts (Table 9). Treatment had no effect on embryo crown rump length, embryo weight, placental weight or uterine weight (Table 9).

Table 9. Potential litter size and reproductive measures for Control and Lupin fed gilts

	Treatment diet	
	Control	Lupin
Day relative to first insemination	28.7 ± 0.26	28.7 ± 0.25
Ovulation rate	15.6 ± 0.41	15.2 ± 0.48
Total luteal weight (g)	5.8 ± 0.25	5.9 ± 0.30
Number of embryos	11.8 ± 0.6*	13.8 ± 0.73*
Embryo Survival (proportion)	0.76 ± 0.04[a]	0.92 ± 0.05[b]
Embryo weight (g)	1.6 ± 0.06	1.6 ± 0.07
Embryo crown-rump length (mm)	25.5 ± 0.40	25.2 ±0.47
Placental weight (g)	27.6 ± 1.8	27.3 ± 2.1
Total uterine weight (g)	3645 ± 218.7	4105 ± 228.9

Within a row, means with different superscripts differ; [ab]P < 0.05; [cd]P = 0.054

4. Application of Research

Overall, the current data partially support the hypothesis that feeding a diet rich in fibre improves oocyte nuclear maturation and embryo survival, confirming previous reports (Ferguson *et al.,* 2007) that dietary modification, specifically the addition of high quantities of fibre to pre-mating diets, can improve oocyte maturity. Importantly, the failure of the bran-based diet to improve oocyte nuclear maturation suggests differences between fibre sources in their ability to affect the ovary. In contrast to previous studies (Ferguson *et al.,* 2006; Ferguson *et al.,* 2007), no changes in reproductive hormones were associated with the improvement in oocyte developmental competence, suggesting the involvement of a direct, gonadotrophin independent mechanism. The current data provides further evidence of a close link between the capacity of oocytes to complete meiosis in vitro and embryo survival in vivo. In support of the data of Ferguson et al., (2007), our results demonstrate that pre-mating diets which improve oocyte meiotic maturation in vitro also increase the number of embryos surviving implantation.

Although comparisons between studies should be treated with caution, the proportion of oocytes reaching metaphase II *in vitro* in the current study is similar to previous reports in the literature (Ferguson *et al.,* 2003; Ferguson *et al.,* 2007). Differences in the age, number of oestrous cycles and genotype of gilts used in the current study compared to those of other studies, as well as differences in *in vitro* maturation systems preclude direct comparisons of dietary effects on nuclear maturation rates. However, it is interesting to note that the improvement in oocyte nuclear maturation in lupin fed gilts (17% compared to controls) is similar to the 10% improvement reported by Ferguson *et al*. (2007) in gilts fed a high fibre diet. The proportion of oocytes reaching MII was 68.2% in the maintenance fed group of Ferguson *et al*. (2003), which is similar to the control group in the present study. In contrast, the increased level of feeding in Ferguson *et al*. (2003) increased nuclear maturation rates to 88% which is similar to the Lupin fed gilts in the current study.

Ferguson *et al*. (2007) suggested the beneficial effects of dietary fibre on oocyte developmental competence was due to the ability of fibre to bind to oestrogens in the intestinal tract resulting in oestrogen being excreted in the faeces and therefore not reabsorbed back into circulation. Interestingly, rabbits fed high fibre diets were shown to excrete three times more oestrogen in the faeces than control fed rabbits (Arias-Álvarez *et al.,* 2009). Reduced circulating oestrogen may reduce the negative feedback effects of oestrogen on the GnRH pulse generator resulting in an increase in LH release as observed by Ferguson *et al*. (2007). Bran was used as a fibre source in the current study due to its high affinity to bind to oestrogen *in vitro* (Arts *et al*. 1991). However, no effects of a bran supplemented diet were observed in the current study. It is possible that the bran diet had a reduced digestibility compared to the Lupin and Control diets and feed, in particular energy, restriction has been shown in a number of studies to inhibit

pulsatile LH secretion (Prunier and Quesnel, 2000; Whisnant and Harrell, 2002). Arts *et al.* (1991) found that wheat bran was the lowest in digestibility (44 ± 1%) in pigs of 12 different fibre sources tested. Graham *et al.* (1986) found that when wheat bran was supplemented at 33% in pig diets, less than 20% of the wheat bran was degraded in the large intestine and faecal output was increased by 127% compared to controls. The high percentage of wheat bran (50%) used in the Bran diet of the current study may have reduced energy availability, as suggested by the lighter body weight of Bran fed gilts at slaughter. However, Ferguson *et al.* (2007) fed a lower feeding rate than what was used in the current study (2.3 kg day^{-1} versus 3 kg day^{-1} respectively) and saw an improvement in oocyte quality in sugar beet pulp supplemented gilts. This indicates that sugar beet pulp is easily digestible and energy levels obtained from the diet are much higher compared to bran.

As well as binding oestrogen in the gut, sugar beet pulp is also rich in non-starch polysaccharides (de Leeuw *et al.,* 2005), a characteristic shared with lupin meal and lupin hulls. Non-starch polysaccharides are digested in the hindgut and increase the amount of volatile fatty acids available as an energy source. Volatile fatty acids help to stabilise interprandial blood glucose and insulin levels (de Leeuw *et al.,* 2005) and Downing *et al.* (1995) found that ewes fed a diet supplemented with lupins had higher insulin levels immediately, and 24 hours, after feeding. A stimulatory effect of insulin on the frequency of GnRH and LH release has previously been reported (Cosgrove and Foxcroft, 1996; Boukhliq and Marting, 1997), equally insulin has been shown to increase the sensitivity of follicle cells to gonadotrophins. Insulin may also directly affect granulosa cell glucose utilisation (Cosgrove and Foxcroft, 1996).

The improvement in oocyte nuclear maturation in the absence of any change in reproductive hormones, suggest a gonadotrophin independent effect of lupins on ovarian function. There are several studies that have shown an effect of diet on follicle-ovary characteristics without affecting circulating gonadotrophin concentrations indicating that metabolic hormones are directly involved in mediating these nutritionally–induced changes in follicular development (Prunier and Quesnel, 2000; Hunter *et al.,* 2004;). The addition of lupins in the experimental diet used in the current study may be stabilising the levels of insulin in the blood. Chang *et al.* (2005) looked at two models of diabetic mice on oocyte quality and follicle development and found that *Akita* type mice which have an autosomal dominant mutation resulting in chronic diabetes (diminished levels of insulin and proinsulin and resultant hyperglycaemia) had smaller oocytes from antral follicles, a delay in nuclear maturation as less oocytes reached GVBD six hours after injection of equine chorionic gonadotrophin, and had fewer antral follicles. They also found female mice with induced acute hyperglycaemia had smaller preovulatory oocytes and reduced nuclear development (Chang *et al.,* 2005). High levels of insulin have been shown to be associated with increased follicular growth (Hunter *et al.,* 2004) by increasing the responsiveness of the ovary to LH (Cosgrove *et al.,* 1992) and through local ovarian mechanisms (Booth

et al., 1996). Cycling gilts supplied with insulin increased ovulation rate irrespective of gonadotrophin secretion (Cox *et al.,* 1987). Additionally, the lupin supplemented diet in the current study was 3 % higher than the Control and Bran diets in protein content. However, previous studies report no improvements in embryo survival (Ferguson *et al.*, 2006) in gilts fed a high protein diet compared to high fibre and high starch fed gilts. Equally, Mejia-Guiderrama *et al.* (2004) reported no effect of protein intake prior to ovulation on the number of ova shed. It is, therefore, unlikely the increased protein intake of lupin fed gilts would have affected oocyte developmental competence.

5. Conclusion

In conclusion, it is apparent from our data that including lupins (35%) in the diets of gilts prior to mating can improve both the quality of the oocytes shed and their capacity to survive implantation in vivo. Considering the majority of potential piglets are lost prior to day 30 of gestation, it seems probable that this improvement in embryo survival will result in more piglets being born. This is particularly important, not just because the observed increase in potential litter size occurred during summer / autumn (i.e. when litter sizes are most likely to be sub-optimal), but also because the inclusion of lupin in gilt diets can easily be implemented at a commercial level. However, given the low level of embryo loss observed in lupin fed gilts (i.e. 10%) compared to what would normally be expected (i.e. 20 – 30%), it is possible that further loss of potential piglets may occur later in gestation (i.e. due to uterine crowding). Consequently, it is imperative that further research is conducted to test the effect of dietary lupins on litter size at term. At a commercial level it is also important to take into account the fact that lupin fibre is not necessarily readily available in the northern parts of Australia. Therefore, future work should identify the mechanism responsible for the improvement in potential litter size in response to lupins, thus making it possible to identify alternative fibre sources which are available throughout the country.

6. Limitations/Risks

There are three limitations to the current data:

1. That the observed improvement in embryo survival may not be translated into more piglets born due to uterine restrictions later in the pregnancy
2. That the observed improvement in embryo survival is only restricted to summer / autumn or the Roseworthy genotype
3. The fact that lupin fibre is only readily available in Western and Southern Australia

7. Recommendations

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

- To conduct further studies designed to determine whether adding lupin fibre to pre-mating diets improves litter size at term, and that this effect is observed throughout the year
- To conduct further studies in weaned sows, in which uterine restrictions on foetal survival are likely to be less severe and therefore improvements in embryo numbers on day 30 of gestation are more likely to be translated into more piglets being born
- To understand the mechanisms responsible for the observed improvement in oocyte developmental competent and embryo survival. Thus enabling the identification of other fibre sources capable of improving litter size.