

# **EVALUATION OF SUPPLEMENTING LONG-CHAIN OMEGA 3 FATTY ACIDS AS A NUTRITIONAL APPROACH TO INCREASE PRODUCTIVITY AND LONGEVITY IN GILTS AND SOWS**

**Report prepared for the  
Co-operative Research Centre for an Internationally  
Competitive Pork Industry**

**By**

**Mr Rob Smits<sup>1</sup> and Dr Megan Mitchell<sup>2</sup>**

<sup>1</sup>Rivalea Australia Pty Ltd., PO Box 78 COROWA NSW 2646

<sup>2</sup>Gamete and Embryo Biology Group, School of Paediatrics and Reproductive Health,  
The University of Adelaide, SA 5005

**July 2009**



Established and supported  
under the Australian  
Government's Cooperative

## Executive Summary

Previous studies overseas has shown that including fish oil at low inclusion levels can increase litter size born in sows when used in corn-soybean diets. However the scientific literature is equivocal in the response of adding long chain polyunsaturated (PUFA) fatty acids on reproduction performance and the mechanisms involved. In Australia, industry use of fish oil supplements in either gilts or sows has also seen variable outcomes. This project aimed to provide a more rigorous assessment of long-chain omega 3 supplementation using fish oil (Salmate™; Feedworks Pty Ltd.) on reproductive performance, lactation performance, follicular fluid composition, follicle cell characteristics and in-vitro oocyte development. In addition, an evaluation of fish oil supplementation on sow retention rate due to possible actions as an anti-inflammatory nutraceutical was conducted in a large-scale field study.

Five experiments were conducted at Rivalea Australia, Corowa and at PPPI, Roseworthy in gilts and lactating sows. In all five experiments, the source of the fish oil used was the same and dietary ingredients were based on wheat and barley cereals with other sources of long-chain omega 3 fatty acids restricted. In all experiments, the dietary analysis and plasma analysis animals for fatty acid profile showed that the long chain omega 3 fatty acids, EPA and DHA were significantly increased with 3 g fish oil/kg diet. Plasma responses increased fairly quickly once supplementation commenced (within 7 days). In most cases, the level of the long chain omega 6 fatty acid, arachidonic acid (ARA) was reduced with elevated DHA and EPA. In gilts, there were no significant increases in litter size born live or total born with fish oil supplementation at 3 g/kg. Farrowing rates were also unaffected. To determine if there was a larger response to a higher rate of supplementation, a second experiment in gilts found that there was again no significant increase in the litter size recovered at 25 days of pregnancy. However, there was some evidence of a response to omega 3 supplementation at 3 g/kg on embryo survival, though this was not significant at 95% confidence. This second study in gilts also showed that increasing the level of fish oil to 10 g/kg did not improve the response. In both gilt studies, the level of fertility was high and further benefits through omega 3 supplementation may have been large enough.

Multiparous sows, on the otherhand, do not currently reach their biological potential. The number of ovulatory follicles often exceeds 20, yet litter sizes of 12-12.5 total born suggest that embryo survival following implantation or foetal survival later in pregnancy compromises sow litter size. When mature sows (parity 5-8) were fed diets with 3 g/kg fish oil during lactation and post-weaning up to mating, we saw a significant increase in embryo survival and a tendency ( $P < 0.06$ ) for more embryos assessed at 23 days post-mating. Embryo survival in these older sows was poorer than what we recorded in gilts (61% vs 82% in Control sows vs gilts) which supports our view that older parity sows are less fertile due to poor embryo survival during peri- and post-implantation (up to 3 weeks post-mating). Ovulation rates were not affected by omega 3 supplementation in sows that averaged 22 corpora lutea in both Control and Omega 3 sows. Concurrent in-vitro

fertilization experiments in multiparous sows showed that early development of the blastocyst was affected by dietary omega 3's from fish oil with a greater rate of cellular division and a higher respiration rate. Analysis of follicular fluid of multiparous sows also confirmed the results in gilts that EPA and DHA in the follicular fluid surrounding the pre-ovulatory follicle are significantly higher, whilst the omega 6 ARA was significantly lower, when omega 3 is added through fish oil.

Although the project has not fully uncovered the mechanisms of how dietary long chain omega 3 fatty acids control affect reproduction processes, we are closer to identifying significant dietary effects on gene activation; on the follicular environment of the pre-ovulatory follicle; and how these might affect embryo survival.

In a field evaluation of the benefits of supplementing with fish oil on sow retention through a possible mode of action as an anti-inflammatory nutraceutical, we demonstrated that there were significantly fewer first-litter sows lost after weaning due to physical causes. Although there tended to be more sows culled for anoestrus after weaning, the net result was for a greater retention and cumulative litter size born to second parity. Sow lameness and high rates of sow mortality is a major problem facing the pig industry and these results provide a non-antibiotic and non-steroid approach to improving the health and welfare of the breeding herd.

In conclusion, the use of low levels of fish oil at 3 g/kg in the diet has been shown to improve embryo survival and potential litter size. The response was higher in multiparous sows compared to gilts, and this may reflect the difference in parities on embryo survival, which was much lower in older sows. The project also provided valuable data as to the reproductive potential of commercial gilts and multiparous sows through measurements of ovulation rate and embryo survival, and we conclude that embryo survival rates in older sows is a key area that needs to be improved to maximize sow litter size. A further field study is proposed to the Pork CRC to evaluate responses on resumption of oestrus, conception rates, pregnancy failure and litter size born following omega 3 supplementation either during lactation and post-weaning or any additive effects by continuing supplementation through early pregnancy.

# Table of Contents

Executive Summary .....	1
Introduction .....	4
Methodology .....	5
Outcomes.....	10
Application of Research.....	34
Conclusion .....	35
Acknowledgements .....	36
Limitations/Risks .....	36
Recommendations .....	36
References .....	37
<b>Appendix 1 - Notes.....</b>	<b>40</b>
<i>Confidential Information.....</i>	<i>40</i>
<i>Deficient Report.....</i>	<i>40</i>
<i>Ownership of Reports.....</i>	<i>40</i>
<b>Appendices.....</b>	<b>41</b>
<i>Appendix 1:.....</i>	<i>41</i>

## Introduction

The importance of essential polyunsaturated fatty acids for foetal development and immunity has been widely known in veterinary health and human nutrition. Recently, the role of omega-3 and omega-6 polyunsaturated fatty acids (PUFA's) has been questioned in breeder diets for sows and boars following commercial improvements in fertility with diets supplemented with fish oils (Reese 2003). Earlier studies have reported that litter size and conception rates are improved when mated gilts and sows are fed a supplemented diet with fish meal (Palmer *et al.* 1970) and fish oil (Perez-Rigau *et al.* 1995). However the role of fatty acids on reproductive function in the pig has not been widely studied and published. Possible mechanisms may be exerted through ovulation rate, oocyte maturation and development competency and/or; embryo survival, due to developmental synchrony or enhanced luteal support during implantation.

Commercial studies reported from overseas have found supplementing corn-soybean diets fed to breeding gilts and sows with fish oil has resulted in commercially significant improvements in litter size born (Spencer *et al.* 2004; Webel *et al.* 2004). However, there are also numerous published studies that have shown no significant response to supplementation in sows (Estienne *et al.* 2006; Perez-Rigau *et al.* 1995; Reese 2003; Rooke *et al.* 2001a; Rooke *et al.* 2001b). Pigs are unable to synthesize unsaturated fatty acids and rely solely on dietary sources. Longer chain fatty acids are derived from the linoleic (c18:2), the precursor for longer chain omega 6 acids, whilst longer chain omega 3 acids can be synthesized from  $\alpha$ -linolenic acid (C18:3). However the rate of synthesis from dietary sources is very low, and plasma responses to only small levels of longer chain fatty acids of either series is far greater than when only the sources of c18 fatty acids are added (Enser 1984). Essential fatty acids have two well-defined roles in reproduction nutrition. They act as precursors to prostaglandins and are structural components of cell membranes. There is little information available from which to make recommendations as to the dietary requirements for essential fatty acids other than linoleic acid (18:2 n-6) and arachidonic acid (ARA, 20:4 n-6). These essential fatty acids are from the omega-6 fatty acid family. Currently there is no published requirement from the omega-3 family, as these are acids are not regarded as essential nutrients for pigs.

As a possible anti-inflammatory, supplementing omega 3 fatty acids may be cost-effective as a nutritional approach to locomotion and lameness problems in piggeries. In companion animal and human health research, supplementation of diets with fish oil is widespread to reduce the incidence of inflammation. Korver and Klasing (1997) stated that dietary fats can also modulate the immune response due to the role of omega 3 fatty acids in chicks. Premature culling due to physical lameness and locomotion accounts for 15% of non-cull for age reasons on commercial farms in Australia and 11% of on-farm destructions (Hughes and Smits 2002). Nutritional management including trace mineral and vitamin supplementation are the focus of proper skeletal development and hoof

formation. However, long-chain omega 3 fatty acids may also be an important nutrient for feed formulation. A large scale commercial study of successive parities formed part of this project to evaluate long-term benefits on sow longevity.

Fish meal has been used for many years in diets fed to breeding pigs as they provide a good source of amino acids and energy. Fish oil is a rich source of the long-chain omega 3 fatty acids eicosapentaenoic acid (EPA; 20:5 n-3) and docosahexaenoic acid (DHA; 22:6, n-3). Recently, legislation governing the inclusion of animal protein, including fish meal, has eliminated this ingredient from sow diets in the EU and UK, whilst a relative high inclusion cost has reduced its use in other countries. In Australia, there has been a recent requirement by AQIS to ban the use of un-refined fish oil in order to protect the domestic aquaculture industry. This has resulted in higher costs of commercial fish oil and possible changes in the relative supply of EPA and DHA.

Sow productivity and herd performance is significantly influenced by the number of pigs born and weaned per sow and productivity per kg of feed used. Through a better understanding of the role of long-chain omega 3 fatty acids and the responsiveness on sow fertility, the pig industry has substantial potential to improve productivity through cost-effective diet formulation. This project evaluated the reproductive response to omega 3 fatty acid supplementation from fish oil in pubertal gilts, lactating sows and gestating sows.

## Methodology

The overall project objective was to quantify the reproductive longevity response between gilts and sows fed diets supplemented with low levels of fish oil compared to un-supplemented control diets. Several experiments were conducted at Rivalea Australia, Corowa, NSW and at the Pig and Poultry Primary Industries (PPPI) research facility at Roseworthy, SA. All omega 3 supplemented diets were formulated and supplied from QAF using cereal based ingredients. Canola meal was excluded from all diets. No vegetable oils were included in any of the diets. Omega 3 fats were supplied from fish oil supplied by Feedworks ([www.feedworks.com.au](http://www.feedworks.com.au)) using salmon oil as the principle source. Other sources of unsaturated fatty acids were excluded from the experimental diets. Saturated fats were supplied in the form of liquid tallow from Barnawartha, Vic.

*Experiment 1: The effect on gilt reproductive performance by supplementing gilt developer diets with omega 3 fatty acids for either 3 or 6 weeks before mating*

Five hundred and seventy pure line and F1 commercial gilts (PrimeGro™ Genetics) were selected for the study at 24 weeks of age. Pubertal gilts were offered diets *ad libitum* as either:

- a basal diet containing no supplementary omega-3 fatty acids from salmon oil (Control) from 24 weeks of age to mating;
- offered a diet with 3 g/kg of fish oil from 24 weeks of age to mating (Omega-3 at 24 weeks);
- offered a diet with 3 g/kg of fish oil from 27 weeks of age to mating (Omega-3 at 27 weeks).

Boars were introduced to each pen of gilts at 24 weeks of age for daily boar stimulation and oestrus detection. Experimental diets were formulated to 14 MJ DE/kg; 142 g crude protein/kg; 0.53 g av/ lysine/MJ DE and were offered *ad libitum* from 23 weeks of age until mating. Tallow was substituted by fish oil in the supplemented diet. Crude fat levels were constant at 56.6 g/kg. The day after mating, all gilts were fed a commercial gestating sow diet formulated to 13 MJ/DE; 146 g crude protein/kg; 0.39 g av. lysine/MJ DE. During pregnancy, all gilts were fed the commercial diet at 2.4 kg/day, which was increased to 2.7 kg from 13 weeks until entry to the farrowing shed at day 112. Thereafter all animals were fed 3 kg a day of a lactation diet (15 MJ DE/kg; 176 g crude protein/kg; 0.51 g av. lysine/MJ DE) until the day after farrowing. Gilts were housed in commercial gilt pens with 12-14 animals per group (1.6 m<sup>2</sup>/gilt) until mating. For a period of six weeks, gilts were individually housed in gestating stalls before moving to group pen accommodation for the remainder of their gestation.

The experimental measurements recorded were live weight and P2 change during supplemental feeding; days to mating; live weight and P2 at mating; oestrus at mating; reason for gilt wastage; farrowing rate; litter size at parity 1.

*Experiment 2a: Evaluation of an increase in the level of fish oil supplementation from 3 to 10 g/on the reproductive response of gilts to omega 3 fatty acids.*

Three hundred F1 commercial gilts (PrimeGro™ Genetics) were allocated to one of three treatments at 24 weeks of age:

- Gilts offered a basal diet with nil omega 3 fatty acid supplementation from fish oil (Control)
- Gilts offered a supplemented diet with 3 g/kg (0.3%) fish oil
- Gilts offered a supplemented diet with 10 g/kg (1.0%) fish oil.

All diets were offered to gilts *ad libitum* from 24 weeks of age to mating and continued on into early pregnancy until slaughter at 25 days post-mating. The diets were formulated to 14.0 MJ DE/kg; 155 g crude protein/kg; and 5.2 g av. lysine/MJ DE. Fish oil was substituted for tallow in each of the fish oil dietary treatments. Crude fat was constant at 5.66 g/kg in all diets. Gilts were housed in groups prior to mating and heat checked with boars. After mating, gilts were housed in gestating stalls until slaughter. Measurements included age at gilt mating; gilt wastage; conception rate; ovulation rate and embryo number and % embryo survival.

*Experiment 2b: Follicular development and oocyte competency in gilts offered diets containing 0 or 10 g fish oil/kg.*

Twenty-four, 18 week old F2 gilts from Roseworthy PPPI piggery, were allocated to one of two treatments;

- Control diet (0g fish oil), or
- Omega-3 diet (10 g fish oil/kg diet) offered diets *ad libitum* for 5 weeks until slaughter.

Gilts were slaughtered at the Murray Bridge abattoir, and ovaries and blood samples were collected at slaughter. For each gilt, ovaries were weighed and oocytes and follicular fluid recovered by aspiration from follicles of two different sizes (Large or Small). Oocytes were then cultured in vitro to enable maturation of the oocyte, whilst granulosa cells were collected and frozen at time 0hr and 24hr (after in vitro culture), with DNA proliferation measured at 24hrs to establish DNA proliferation rate as an indicator of mitosis within the follicular environment. Following in vitro culture of immature oocytes for 46 hr, mitochondrial activity and nuclear maturation rates (orcein staining) were determined for individual oocytes. Plasma and follicular fluid fatty acid analysis was conducted. The aim of this experiment was to understand how dietary treatment affected the processes in the follicular environment and oocyte quality before fertilization.

*Experiment 3a: The effect of omega 3 supplementation with 3g fish oil/kg in mature sows during lactation on post-weaning reproductive performance.*

One hundred and eighty F1 commercial sows (PrimeGro™ Genetics) ranging in parity between 4 to 7 prior to farrowing were allocated to one of two treatments at farrowing:

- Sows offered a basal diet with nil omega 3 fatty acid supplementation from fish oil (Control)
- Sows offered a supplemented diet with 3 g/kg (0.3%) fish oil during lactation and up to mating.

Using a commercial lactation diet specification formulated to 14.9 MJ DE/kg; 186 g crude protein/kg; 0.50 g av. lysine/MJ DE, a base diet was formulated and fish oil was substituted for tallow in the supplemented diet. Sows were offered the treatment diets to appetite up to four times a day. At 26.5 days after farrowing, sows were weaned and continued on their treatment diet fed daily at 3 kg until mating. Thereafter, mated sows were transferred to individual gestation stalls and fed 2.7 kg of a commercial gestating diet (13 MJ DE/kg; 135 g crude protein/kg; 0.4 g av. lysine/MJ DE) once a day until slaughter at 23 days post-mating. Measurements included lactation performance, live weight and backfat P2 changes over lactation; weaning to oestrus interval; conception rate; ovulation rate; embryo number and % embryo survival.

*Experiment 3b - Omega-3 FA supplementation of sows will alter oocyte metabolism and thus affect oocyte competence and embryo quality.*

Six replicate experiments, comprising n=5 sows per diet (Control or Omega-3 diet formulated with 3 g fish oil/kg diet and fed for 6 weeks, including the pre-farrowing period (6 days), during lactation when sows were offered diets *ad libitum* and post-weaning. From a total of 30 sows per dietary treatment, ovaries were collected 3-5 days post-weaning from sows slaughtered at Laverton, Victoria. For each sow, ovarian weight was measured, and oocytes aspirated from 3 different follicle size groups (1-4mm, 4-8mm and 8+mm). Oocytes were matured in vitro for 44-46hr, and then measured (see below) or fertilized using an IVF procedure.

Fertilized embryos were developed in vitro for 6 days, and development rate, cell number and metabolism determined at the blastocyst stage. Individual embryos were measured following in vitro fertilization for: 1) oxygen consumption, using Embryoscope equipment, 2) mitochondrial distribution and calcium levels, determined using dual mitochondria-specific dyes and confocal microscopy, 3) glucose consumption, using a microfluorometric assay. Follicular fluid and granulosa cells were also collected during aspiration for fatty acid analysis and the determination of expression levels of genes involved in steroid hormone and prostaglandin function, respectively.

These experiments aimed to ascertain whether Omega-3 supplementation will improve fertilization and early embryo cleavage rate, and the mechanism involved in this effect.

*Experiment 4: The effect of omega 3 supplementation with 3g fish oil/kg continuously for two parities on reproductive performance and longevity*

Nineteen hundred and fifty eight F1 commercial gilts (PrimeGro™ Genetics) were allocated to one of two treatments the day after mating:

- Sows offered a basal diet with nil omega 3 fatty acid supplementation from fish oil (Un-supplemented Control)
- Sows offered supplemented diets with fish oil during 1<sup>st</sup> gestation, lactation and second parity gestation. The diets were supplemented with 6 g fish oil/kg during gestation and 3 g/kg during lactation.

Mated gilts were allocated to dietary treatment within 2 days after mating. Gilts were individually housed in stalls for 6 weeks then transferred to pens and housed in groups of 8-10 on partially slatted concrete floors (1.65 m<sup>2</sup>/sow). Feeding levels were restricted to 2.7 kg for the first 13 weeks, then increased to 3.2 kg for the last two weeks in group pens. On 112 days of gestation, sows were moved into farrowing crates and introduced to either the un-supplemented or supplemented lactation diets as a

continuation of their dietary treatment. Following weaning, all sows were fed their gestation treatment diet *ad libitum* and housed in group pens of approximately 20 sows on partially slatted concrete floors. After two weeks, sows were re-introduced to the boar shed and mated on their second post-weaned oestrus as described as a skip-a-heat mating by Clowes *et al.* (1994). After mating, parity 1 sows were stalled individually and fed 2.7 kg a day of their treatment gestation diet. After six weeks, sows were group-housed in pens of 8-10 for the remainder of their gestation until re-entering the farrowing accommodation at day 112.

Data was collected from the herd recording system at Rivalea Australia including the reproductive performance, cause of reproductive failure and overall productivity over two parities from the number of gilts mated allocated at the start of the treatment period.

## Outcomes

### *The effect of dietary omega 3 supplementation on plasma levels of omega 3 and omega 6 fatty acids*

The response to supplementation with fish oil on the long-chain omega fatty acids of EPA and DHA was significantly observed in plasma samples analyzed from pubertal gilts (Figure 1). The gilts fed the control diets for the first 21 days had consistently lower levels of EPA and DHA over the three week period compared to the Omega 3 treatment that commenced at 24 weeks of age ( $P < 0.001$ ).

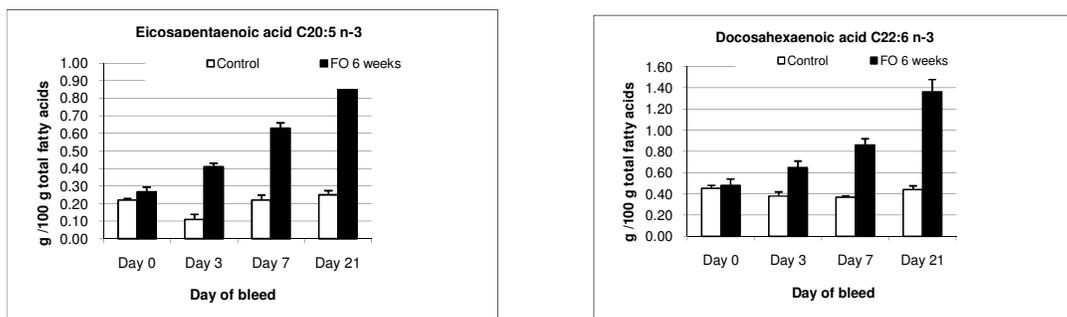


Figure 1. The plasma levels of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) after 0, 3, 7 and 21 days to supplemental feeding of Omega 3 fatty acids in plasma of unmatred gilts between 24 and 27 weeks of age (Control □ ; Omega 3 at 24 weeks ■).

Omega 3 supplementation with fish oil did not affect the plasma levels of linoleic (omega 6) or  $\alpha$ -linolenic acid (omega 3) (Figure 2). This was expected as the level of biosynthesis between C18 essential fatty acids and longer chain fats is generally considered to be low (Azain 2004). The plasma results showed clearly that supplementation with only low levels of dietary omega 3 fatty acids from fish oil can have a large impact on circulating long-chain fatty acids without altering the levels of shorter chain pre-cursor fatty acids. Therefore it is possible to manipulate the fatty acid profile through the diet.

In experiment 1, the mean $\pm$ SE live weight of gilts at 24 weeks of age was 108 $\pm$ 0.41 kg with 11.8 $\pm$ 0.1 mm P2 backfat. By mating at approximately 209 days of age, live weight and P2 remained similar between treatments (145.0 $\pm$ 0.90 kg; 14.8 $\pm$ 0.19 mm).

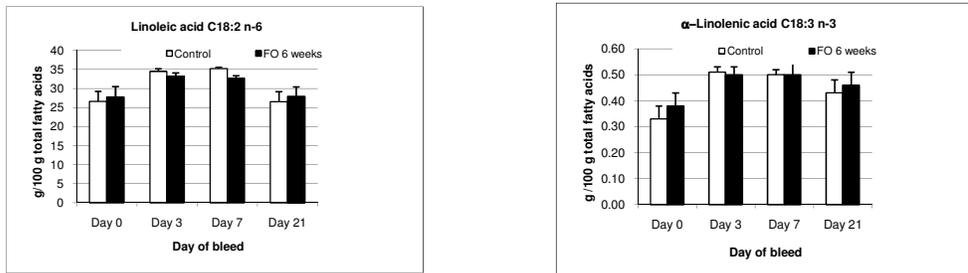


Figure 2. The plasma levels linoleic and  $\alpha$ -linolenic acid (g/100 g fat) after 0, 3, 7 and 21 days to supplemental feeding of Omega 3 fatty acids in plasma of unmatred gilts between 24 and 27 weeks of age (Control  $\square$  ; Omega 3 at 24 weeks  $\blacksquare$ ) from Experiment 1.

### *Pubertal response to supplementation*

There were no treatment effects on gilt cycling or gilt removals from the breeding herd prior to mating (Table 1). There was a significant treatment difference on age at mating with gilts fed the omega-3 diet from 24 weeks of age mated older ( $P < 0.05$ ). This appeared to correspond to a delay in the onset of puberty, measured as the days taken to cycle. The shorter duration of supplemental omega-3 feeding had an intermediate response.

The majority of gilts were mated on their second oestrus. Of the Controls, 120 of the 154 gilts mated (77.9%) occurred on 2<sup>nd</sup> or 3<sup>rd</sup> detected oestrus compared to 109/147 (74.1%) gilts on the Omega-3 diet at 24 weeks of age and 112/154 (72.7%) gilts on the Omega-3 diet at 27 weeks of age. There were no significant differences in the proportion of gilts mated on 2<sup>nd</sup> or 3<sup>rd</sup> oestrus between treatments ( $\chi^2$  1.18;  $P = 0.554$ ). The mean duration of omega-3 fatty acids in the gilts allocated to Omega-3 at 27 weeks of age was  $23.7 \pm 1.29$  days, whilst the mean duration on the supplemented diet in gilts on Omega-3 at 24 weeks was  $46.9 \pm 1.30$  days by the time mating ( $P < 0.001$ ).

The proportion of animals removed from the herd was attributed to reproductive and physical causes. From all treatments, poor structural conformation ('Feet and Legs') described 40.7% of the 108 removals, whilst 7.4% removals were classed as poor body condition ('Condition'). Of the total removals, 56 gilts (51.8%) were classed as anoestrus ('Stale'), however 13 of these were recorded to have displayed a Standing Heat Response but were not detected to cycle again (failed to re-cycle or silent oestrus).

Table 1. The effect in gilts of fish oil supplementation fed from 24 or 27 weeks of age on the incidence of puberty response to boars, days taken to cycle and the age at mating (mean±SE) -Experiment 1).

	No. gilts cycled <sup>1</sup>	No. culled for anoestrous	Total removed before mating <sup>1</sup>	Days taken to cycle	Age at mating (days)
Control	159/187 (85.0)	14/187 (7.5%)	33/187 (17.6)	25.7±1.75	213.1±1.25 <sup>a</sup>
Omega 3 Long duration	159/186 (85.5)	15/186 (8.1%)	39/186 (21.0)	29.4±1.70	217.7±1.28 <sup>b</sup>
Omega 3 Short duration	160/190 (84.2) $\chi^2=0.12$	14/190 (7.4%) $\chi^2=0.07$	36/190 (18.9) $\chi^2=0.67$	27.7±1.76	214.6±1.26 <sup>ab</sup>
P value	0.941	0.964	0.714	0.325	0.033

<sup>1</sup>Determined as observed standing heat response in the presence of a mature boar.

<sup>ab</sup>Mean values within column with different superscripts differ significantly P<0.05.

#### *First parity reproductive performance - Experiment 1*

Reproductive performance from mated gilts is summarized in Table 2. There was no significant effect of supplementing omega-3 fatty acids on reproductive performance either in gilts supplemented from 24 weeks of age or 27 weeks of age. Although the data was collected from gilts mated between July to December, the effect of season on farrowing rate and litter size was not a significant co-variable in the analysis (P>0.50). All treatments performed at a high level and were reflective of the commercial performance from the piggery during this time of the year.

Table 2. Farrowing rates (sows successfully farrowed from 1<sup>st</sup> mating) and litter size<sup>1</sup> (mean±SE) of gilts following supplementation with Omega-3 PUFAs from 24 or 27 weeks of age prior to mating or no supplementation (Control) - Experiment 1.

	No. gilts mated	Farrow rate (% of mated)	Litter size born live	Litter size total born	Still births	Still birth (% of total)
Control	154	130/154 (84.4)	10.5±0.24	11.6±0.24	1.1±0.13	9.0±1.08
Omega-3 Long duration	147	128/147 (87.1)	11.0±0.24	11.9±0.24	0.9±0.12	7.5±1.14
Omega-3 Short duration	154	136/154 (88.3) $\chi^2=1.05$	10.7±0.24	11.6±0.25	0.9±0.13	7.7±1.16
P value		0.592	0.325	0.527	0.621	0.573

## *Ovarian and embryo survival response to increasing levels of supplementation of omega 3 fatty acids in gilts - Experiment 2a*

In experiment 2, we tested the hypothesis that gilts would respond to a higher level of supplementation from 3 to 10 g fish oil/kg. Supplementation occurred from 24 weeks of age through to pregnancy when gilts were slaughtered at 25 days post-mating. The mean $\pm$ SE live weight of gilts at 24 weeks of age was 104.5 $\pm$ 0.5 kg with 10.3 $\pm$ 0.1 mm P2 backfat. At mating, live weight (150.0 v 148.6 kg) and P2 (14.3 v 14.3 mm) were similar between Controls and Omega 3 g/kg but were slightly higher in Omega 10g/kg (153.9 kg and 15.1 mm;  $P < 0.05$ ). However during the period of *ad libitum* feeding of dietary treatments, the weight gain between 24 weeks of age to mating was unaffected by treatment (0.851 $\pm$ 0.012 kg/d;  $P = 0.573$ ). Gilts allocated to the higher supplementation level of Omega 10 g/kg tended ( $P < 0.10$ ) to be older at mating (225.0  $\pm$ 1.12 days of age) compared to the Omega 3 g/kg or Control gilts (221.5 $\pm$ 1.19 and 221.8 $\pm$ 1.26 days). Unlike the first experiment, the age at oestrus onset was not recorded. Gilts were not exposed to boar stimulation until 29 weeks of age and mated on their first observed standing oestrus. The oestrus at mating was not recorded, but given the late exposure to boars, it is likely that the majority of gilts would have been mated on 1<sup>st</sup> oestrus. By slaughter at 25.2 $\pm$ 0.1 days post-mating, the gilts averaged 161 $\pm$ 1.0 kg live weight and 16.3 $\pm$ 0.3 mm P2. There was no significant treatment effect on gestation weight or fatness by slaughter.

Table 3 summarizes the reproductive performance from Experiment 2 when gilts were supplemented with 0, 3 or 10 g fish oil/kg from 24 weeks of age. There was no increase in response to a higher level of supplementation on the proportion of gilts that were mated, nor on conception rate. The treatment effect on ovulation rate (number of ovarian corpora lutea in total) was not significant, and post-hoc tests between Omega 3 g/kg and the Controls, probability (P) values were greater than 0.10. Embryo recovery at 25 days of gestation was similar between treatments. When expressed as a percentage of corpora lutea, the percentage embryo survival tended to be affected by dietary treatment, although the post-hoc comparisons were not statistically significant (Control v Omega 3 g/kg,  $P = 0.213$ ). The effect of increasing omega 3 supplementation above 3 g/kg did not improve embryo survival (Control v Omega 10 g/kg,  $P = 0.764$ ; Omega 3 g/kg v Omega 10 g/kg,  $P = 0.596$ ). Although the conception rates were commercially high, the proportion of the gilt pool mated was low. The main cause of reproductive failure was anoestrus (30 % of entered gilts at 24 weeks of age) which was considerably higher than reported in experiment 1 when boars were used to stimulate gilts. In Experiment 2 we decided to delay the start of boar stimulation to avoid any non-dietary treatment differences that may occur between boars on the onset of puberty. Anoestrus was unaffected by dietary treatment ( $P = 0.728$ ).

There have been no published studies to date where different levels of supplementation on fecundity in sows have been evaluated. The results of this

experiment provide the first evidence that there is not a positive linear response on embryo survival or litter size by early gestation to omega-3 supplementation between 3 and 10 g/kg. Plasma fatty acid levels were recorded on a subset of gilts between 24 weeks of age and 2 days after mating and at 25 days of gestation. The EPA and DHA concentrations by slaughter at day 25 gestation in the Control were 0.42 g EPA/100 g fatty acids and 1.35 g DHA/100 g fatty acids. Dietary supplementation at 3 and 10 g fish oil/kg substantially increased plasma levels of EPA (0.89 and 2.25 g/100 g fatty acids, respectively) and DHA (2.24 and 4.15 g/100 g fatty acids). The level of the omega 6 arachidonic acid (ARA) in sow plasma was significantly lower in Omega 3 g/kg gilts at 25 days of gestation compared to the Controls (9.2 v 12.9 g/100 g total fatty acids,  $P < 0.05$ ).

As a result of Experiment 1 and 2 in gilts where benefits were not clearly evident, it was decided not to continue investigation into the role of omega 3 fatty acid supplementation in the pubertal gilt.

Table 3. Reproductive performance due to increasing omega 3 supplementation levels from entry at 24 weeks of age to day 25 of gestation from Experiment 2a.

Treatment	No. allocated	Gilt pool mated	Conception rate	Ovulation rate	Embryo count	Embryo survival %
Control	114	66.7% (76/114)	88.2% (67/76)	16.9±0.33	13.7±0.43	81.8±2.01
3 g/kg fish oil	120	61.7% (74/120)	84.3% (59/70)	15.9±0.38	14.1±0.49	88.9±2.32
10 g/kg fish oil	122	63.9% (78/122)	91.4% (64/70)	16.8±0.34	14.1±0.44	84.3±2.06
P value		$\chi^2$ 0.64, P=0.728	$\chi^2$ 1.69, P=0.429	0.113	0.775	0.070

*Follicular development and oocyte competency assessment in gilts offered omega 3 fatty acids through fish oil supplementation at 10 g/kg - Experiment 2b (Roseworthy)*

There was no effect of supplementation at 10g fish oil/kg on average final body weight of gilts (97±2.1 and 95±2.2,  $p=0.5$ , for gilts fed omega-3 or control diets). In this pilot study, there was no significant effect of diet on the distribution of ovarian follicles as small (<3mm) or large sizes (>4mm) (Table 4). However, for larger follicles from Omega 3 treated gilts there was significantly greater oocyte maturation ( $p=0.027$ ) compared to Controls, whilst for in oocytes derived from smaller follicles of Omega 3 gilts there were significantly less oocyte maturation ( $p=0.004$ ). Oocytes during ovulation come from the larger follicles, so this increase in in-vitro maturation within the large follicles of Omega 3 treated gilts suggests that omega 3 supplementation advances oocyte maturation.

Table 4. The evaluation of follicle distribution and oocyte maturation in oocytes derived from pre-pubertal gilts (23 weeks of age) fed un-supplemented diets (Control) or supplemented with fish oil omega 3 fatty acids (10g fish oil/kg diet).

	Average number of follicles		Oocyte maturation			
	Small	Large	Large		Small	
			<u>Mature</u>	<u>Immature</u>	<u>Mature</u>	<u>Immature</u>
Control	84.3	28.9	63 <sup>a</sup>	37 <sup>d</sup>	55 <sup>d</sup>	45 <sup>a</sup>
High Omega 3	90.3	34.3	75 <sup>b</sup>	25 <sup>c</sup>	21 <sup>c</sup>	78 <sup>b</sup>

Different superscripts (a, b) within columns differ  $p < 0.05$ . Different superscripts (c, d) within columns differ  $p < 0.004$ . Chi-square statistical analysis.  $N > 51$  oocytes per diet for each follicle size.

Diet did not affect the total concentration of omega-6 fatty acids in the plasma, but in the follicular fluid itself, gilts fed the omega-3 enriched diet had significantly less total omega-6 fatty acids surrounding the oocyte ( $P < 0.001$ ). However, in general the concentration of omega-3 and omega-6 fatty acids in follicular fluid was generally similar to that contained within the plasma (Table 5).

Table 5. Levels of fatty acids (as % total fatty acids) in ovarian follicle fluid and plasma from gilts fed Control or Omega-3 enriched diet

	Follicular Fluid		P value	Plasma		P value
	<i>Control</i>	<i>Omega-3</i>		<i>Control</i>	<i>Omega-3</i>	
Total Monounsats.	24.4±0.43	25.5±0.43	0.09	21.9±0.22	21.1±0.38	0.09
Total Saturates	40.6±0.24	42.2±0.24	<0.001	35.5±0.23	34.3±0.40	0.03
Total n-6	31.0±0.50	25.8±0.50	<0.001	37.9±0.25	37.0±0.43	0.11
Total n-3	3.2±0.13	5.8±0.13	<0.001	3.8±0.15	7.0±0.26	<0.001
ARA	12.4±0.038	9.8±0.38	<0.001	9.5±0.19	7.7±0.33	<0.001
DHA	1.5±0.08	2.7±0.08	<0.001	1.5±0.06	3.0±0.11	<0.001
EPA	0.3±0.05	1.0±0.05	<0.001	0.4±0.02	1.7±0.04	<0.001

### *Ovarian and embryo survival response to omega 3 supplementation during lactation and post-weaning in multiparous - Experiment 3a*

Previous field studies at Rivalea Australia (Smits unpublished) and overseas (Palmer *et al.* 1970; Webel *et al.* 2004) have reported that subsequent litter size was increased when sows were offered a supplemented diet with omega 3 derived from fish oil during the previous lactation. However there has also been reports published where fish oil was supplemented to either gestation diets and lactation (Rooke *et al.* 2001b) or post-weaning diets alone (Perez-Rigau *et al.* 1995) there was no effect on subsequent fertility. This experiment was designed to test the hypothesis that supplementation with 3 g fish oil/kg during lactation and pre-mating would increase litter size through early embryo survival.

Two hundred and forty seven multiparous sows ( $5.78 \pm 0.07$ ) were allocated on entry to the farrowing house at Day 109 of gestation based on lifetime average litter size and parity. Dietary treatments were fed on entry to the farrowing at 3 kg/day. The base diet was formulated with 14.9 MJ DE/kg, 186 g crude protein and 9 g lysine/kg. In the omega 3 supplemented diet, fish oil replaced tallow. Fifteen sows were excluded from the study data set due to incomplete records, low litter weight gains, mortality. In the remaining dataset, there were 114 Control sows and 118 Omega 3 sows.

The mean live weight and backfat P2 post-farrowing was  $294.3 \pm 1.96$  and  $15.6 \pm 0.31$  mm. The mean parity and distribution at farrowing was similar between the treatments ( $5.79 \pm 0.08$ ). From Day 1 post-farrowing, sows were offered their treatment diet *ad libitum*. Daily feed intake averaged  $7.8 \pm 0.05$  kg over 26.5 days. Litter size nursed was equalized between treatments with an average of  $10.1 \pm 0.08$  piglets. Lactation performance was unaffected by dietary feeding from Day 109 of gestation followed by lactation supplementation with omega 3 fatty acids from fish oil (Table 6). Weaning weight was similar between Control and Omega 3 litters when measured at 25.5 days of age ( $7.14 \pm 0.13$  kg v  $7.16 \pm 0.12$  kg,  $P=0.922$ ). The number of piglets weaned was also unaffected by diet ( $8.3 \pm 0.20$  v  $8.2 \pm 0.18$ , respectively:  $P=0.734$ ). Sow daily intake was similarly unaffected by diet ( $7.8 \pm 0.08$  v  $7.7 \pm 0.07$  kg/d;  $P=0.318$ ). Although there was no significant treatment difference, it is noteworthy that these mature sows recorded some weight loss during lactation, with minor loss of backfat P2.

Table 6. Lactation performance of mature sows (parity 5-8) offered diets supplemented with 3 g fish oil/kg or 0 g fish oil/kg (Control) during lactation (Experiment 3).

Treatment	Piglet weight Day 1 (kg)	Piglet weight gain (kg/d)	Litter weight gain (kg/d)	Lact. Weight loss (kg)	Lact. P2 loss (mm)
Control	$1.48 \pm 0.03$	$0.222 \pm 0.005$	$1.73 \pm 0.063$	$13.1 \pm 1.87$	$0.5 \pm 0.53$
3 g/kg fish oil	$1.53 \pm 0.03$	$0.220 \pm 0.004$	$1.72 \pm 0.057$	$12.9 \pm 1.76$	$0.3 \pm 0.48$
P value	0.207	0.839	0.950	0.957	0.743

Following weaning all sows were housed in individual stalls until mating by artificial insemination at first observed oestrus (am) and 24 hours later. During this time, all sows were fed their respective treatment diet fed during lactation at 3 kg/day. Once mated, all sows were offered the same un-supplemented commercial gestation diet (12.9 MJ DE/kg; 140 g crude protein/kg; 6.2 g lysine/kg) which contained no fish oil and were housed in individual stalls in a gestating facility. At 23.3±0.08 days post-mating, sows were slaughtered at a commercial abattoir and reproductive tracts were recovered. Pregnancy rates, embryo number, ovulation rate and embryo survival were recorded (Table 7).

Table 7. Reproductive performance of mature sows (parity 5-8) following dietary supplementation with 3 g fish oil/kg offered during lactation (23 days) and from weaning to mating (Experiment 3).

Treatment	Weaning to oestrus (d)	Pregnancy rate	Ovulation rate <sup>1</sup>	Embryo count <sup>1</sup>	Embryo survival % <sup>1</sup>
Control	5.3±0.27	56.7% (51/90)	22.2±0.66	13.6±0.79 <sup>x</sup>	61.5±3.42 <sup>x</sup>
3 g/kg fish oil	5.0±0.24	63.3% (57/90)	22.2±0.52	15.5±0.62 <sup>y</sup>	70.0±2.68 <sup>y</sup>
P value	0.433	$\chi^2$ 0.83, P=0.361	0.995	0.055	0.054

<sup>1</sup>LSM value with lifetime average total born (11.2) included in GLM model as a significant (P<0.05) co-variate factor. <sup>xy</sup>Mean values within columns differ significantly (P<0.10).

There was no effect of omega 3 dietary supplementation from fish oil on the resumption of oestrus conception rate or ovulation rate. Pregnancy rates were low, though not surprising given the age of the sows used in the experiment. There was a statistically significant increase in embryo number (P<0.06) and embryo survival with supplementation at 3 g fish oil/kg. Unfortunately, the variability in litter size after implantation was high between time block replicates, with a coefficient of variation of 29.6%. The raw data means±standard deviation for litter size was 14.4±3.76 and 14.8±4.9 for Controls (n=50) and Fish oil (n=57), respectively. The raw data means±standard deviation for embryo survival was 63.6±16.8% and 66.7±19.8% for Controls (n=50) and Fish oil (n=57), respectively.

*Omega 3 fatty acids affect the follicular environment, oocyte metabolism and embryo development in-vitro - Experiment 3b*

For this experiment, sows were fed 3g fish oil per kg diet or a control diet, a rate that was lower than that fed to gilts in Experiment 2b. Sows were fed the supplemented diet during

lactation and up to the ovulation. At day 4 post-weaning, unmated sows were slaughtered and their reproductive tracts collected for ovarian assessment, oocyte collection and in-vitro metabolism measurements.

There was no significant difference in the total concentration of omega-3 or omega-6 fatty acids, however, in follicular fluid from the omega-3 fed sows, arachidonic acid was lower ( $p=0.02$ ) and the long-chain omega-3 PUFA DHA was higher ( $p=0.03$ ) (Table 8). Of interest, there was significantly lower concentrations of total omega-3, EPA and DHA in small follicles (1-4mm) compared to larger, 4-8mm and 8+mm follicles ( $p<0.05$ , data not shown).

Table 8. Fatty acid concentration in follicular fluid from sows fed Control or Omega-3 enriched diet

	Control	Omega-3	p-value
Total Monounsaturates	25.1 ± 0.33	25.2 ± 0.37	ns
Total Saturates	39.2 ± 0.38	40.0 ± 0.42	ns
Total omega-6	30.6 ± 1.88	26.1 ± 2.09	ns
Total omega-3	3.8 ± 0.27	4.0 ± 0.30	ns
Arachidonic acid	11.2 ± 0.26	10.2 ± 0.29	0.02
DHA	0.8 ± 0.10	1.1 ± 0.11	0.03
EPA	0.5 ± 0.04	0.5 ± 0.04	ns

N=10-11 samples per treatment over four replicates. Samples taken from all follicle sizes.

Follicle size was measured on a subset of ovaries taken over six weekly replicates from a total of 30 sows per treatment. There was no significant effect of the dietary supplementation of Omega-3 fatty acids on the distribution of follicle size on recovered ovaries, however there tended to be more medium and large follicles for the Omega-3 fed group (Figure 3). This was a similar outcome as seen in pre-pubertal gilts in Experiment 2b.

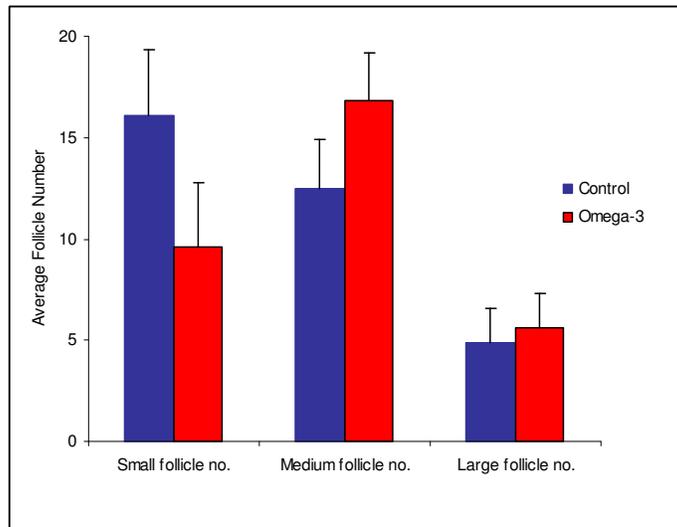


Figure 3: Average number of follicles (small 1-4 mm, medium 4-8 mm and large >8mm) per ovary, for sows fed Control or Omega-3 enriched diet.

Following maturation in-vitro, oocytes underwent in vitro fertilisation (IVF) and subsequent embryos were assessed and cultured to day 6 of development. There was no effect of diet prior to ovulation on the proportion of oocytes that were fertilised and cleaved (Figure 4a). However, sows fed Omega 3 diets had a significantly higher embryo development rate to the blastocyst stage (Figure 4b). Furthermore, there tended to be an increase in the number of cells in these blastocysts when assessed at a defined post-fertilisation timepoint in the Omega 3 sows (data not shown,  $p=0.059$ ).

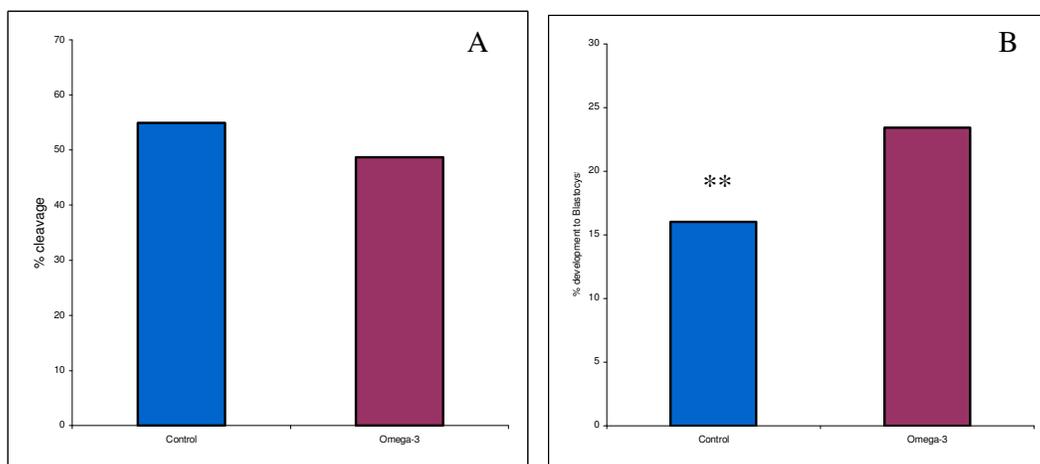


Figure 4 Fertilisation (A) and embryo development rate (B) of oocytes recovered from sows fed control or omega-3 enriched diet and fertilised in vitro. 185 embryos were cultured across 6 replicate experiments, \*\* Dietary response significantly different at  $p<0.05$

To endeavour to understand how dietary omega-3 fatty acids might influence the quality of the oocyte and subsequent embryo development, we examined embryo metabolism and the expression of specific genes in the follicular cells (granulosa cells) that contribute to follicular steroids and prostaglandins.

Oxygen is consumed by embryos to enable the utilisation and production of energy for embryo development. Eighteen zygotes (single-cell stage after fertilization) per treatment were taken from two experimental replicates and assessed for oxygen consumption as an indication of respiration rate. At the single-cell embryo stage (zygote), dietary Omega-3 enrichment did not influence embryo respiration rate significantly (Figure 5A). As the embryo grows, its reliance on glucose as an energy substrate increases. To assess the glucose metabolism by the blastocyst stage, 112-144 blastocysts per treatment collected over four experimental replicates were assessed. There was a significant reduction in glucose utilisation rate by blastocyst stage in the cultured embryos from Omega 3 sows compared to the Controls ( $p < 0.006$ , Figure 5B).

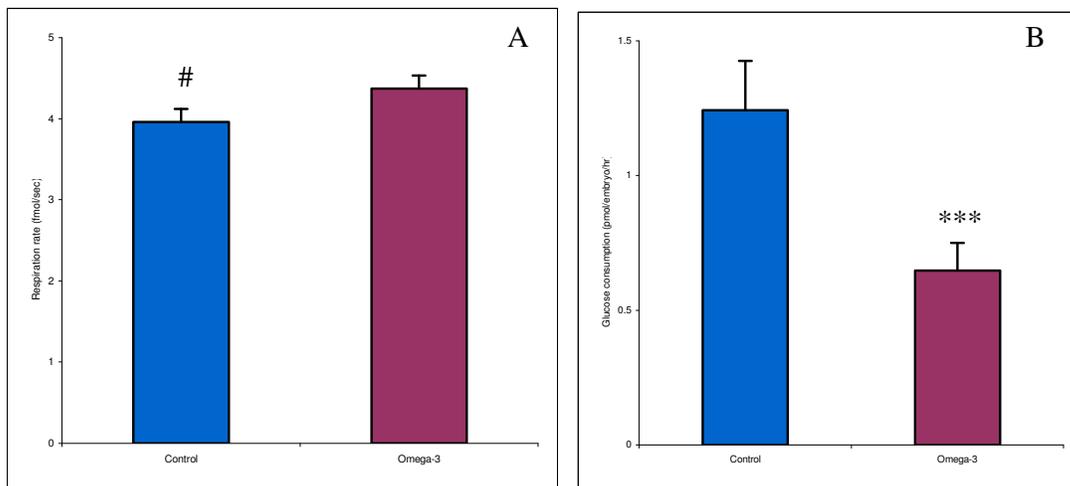


Figure 5. The effect of dietary omega-3 fatty acid supplementation to sows on embryo metabolism. A) Zygote oxygen consumption (respiration rate) and B) Blastocyst glucose metabolism. # indicates  $p = 0.09$ . \*\*\* Dietary response significantly different at  $p < 0.006$ .

There was no significant difference in the expression level of the steroid hormone oestrogen receptor (ER2- $\alpha$ ) or follicle stimulating hormone receptor (FSH-R), but there tended to be reduced expression of the progesterone receptor (PR) in granulosa cells from sows fed the Omega-3 enriched diet ( $p < 0.07$ , Figure 6A). Likewise, although expression of the prostaglandin receptors EP2 and EP4 was unchanged by diet, expression of cyclooxygenase-2 (COX-2), which regulates PGF2 $\alpha$ , tended to be less in granulosa cells from sows fed the Omega-3 enriched diet ( $p < 0.07$ , Figure 6B).

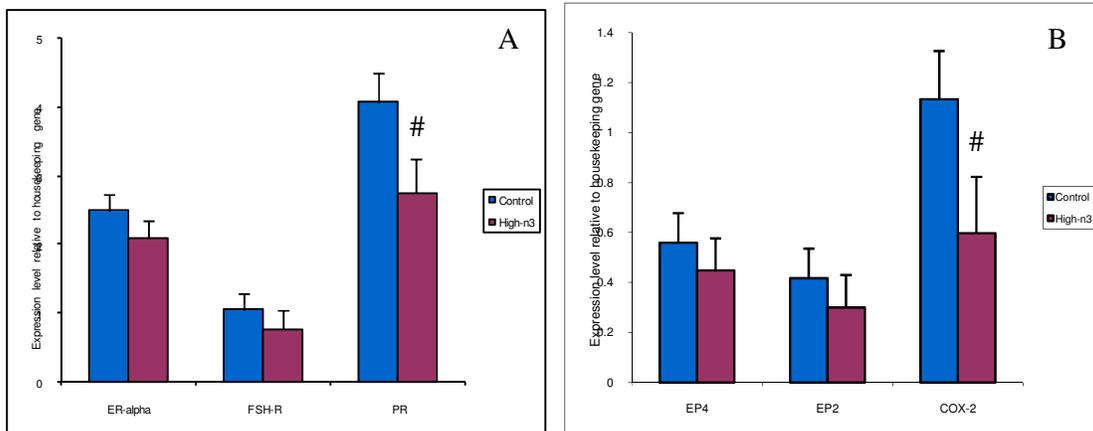


Figure 6. Gene expression in granulosa cells from ovarian follicles of Control or Omega-3 fed sows, for A) estrogen receptor- $\alpha$  (ER-  $\alpha$ ), follicle stimulating hormone receptor (FSH-R) and progesterone receptor (PR), and B) prostaglandin receptors EP4 and EP2, and cyclooxygenase-2 (COX-2). # Dietary response significantly different at  $p < 0.07$

In summary, we found that the mature sows fed Omega 3 supplemented diets using fish oil at 3 g/kg of the diet had a changed follicular development with more follicles in the medium to large size and a lower concentration of arachidonic acid. These confirm the findings in gilts. Using in-vitro fertilised oocytes and measurements of metabolism, we showed that there was a greater rate of cellular development to blastocyst stage and this was supported by measures of a higher respiration rate, however the rate of glucose utilization was significantly reduced. Finally, the measures of gene expression showed that diet affected gene activation from follicular granulosa cells, with a reduction in COX-2 and progesterone receptors which are involved in prostaglandin synthesis.

#### *Long term effects of supplementation of omega 3 fatty acids from fish oil on reproductive performance and culling rates in young sows - Experiment 4*

Early losses in sow herds due to locomotor issues and mortality are variable, however we have reported 12% of non-age related culling is due to structural or skeletal problems. Sudden death generally attributable to abdominal accident (gastric torsions) or heart failure account for 61% of mortalities and 29% of mortalities are sows euthanased due to irreparable injury related to skeletal problems or chronic lameness. This experiment evaluated the response to supplemented omega 3 fatty acids as salmon oil in the breeding gilt and sow (formulated to supply approximately 18 g fish oil/day) as a possible non-medicated sow retention strategy.

Over 20 weeks of allocation, 1958 gilts at a mating age of  $224 \pm 0.25$  days were allocated in equal numbers each week to a combination of breeder diets over two parities

with either supplemented omega 3 fatty acids from fish oil or un-supplemented (Control). Before commencing the study, all gilts were fed diets un-supplemented with fish oil.

Reproductive performance for gilts and sows on the supplemented and un-supplemented dietary regimens is summarized in Table 9. There was no significant treatment difference in the number farrowing rate, gestation length or litter size born in the first parity. Litter size and farrowing rate were high and weekly block effects were non-significant, indicating a low variability in fertility over the 20 weeks of allocation which included the summer-autumn mating period. The average weaning to re-mating interval was significantly lower in the sows fed the Omega-3 supplemented feeding regimen ( $P < 0.05$ ). The long WMI values were due to the management practice of mating on skip-a-heat mating on the 2<sup>nd</sup> oestrus after weaning. A criterion of 28 days was used to further assess the treatment response on post-weaning resumption of oestrus. This was nominated based on 7 days post-weaning oestrus (1<sup>st</sup> oestrus) plus 21 days. Even though the difference between supplemented and un-supplemented sows in the average weaning to re-mating interval was small (a difference of only 0.7 days), the proportion of sows re-bred within the expected timeframe of 28 days post-weaning was 6% higher in the Omega-3 sows ( $P < 0.05$ ). The cumulative litter size born live for parity 1 and 2 tended to be higher in sows fed Omega-3 supplemented diets. This data included piglets born live to both parities from return matings. At 80% confidence, the difference of half a piglet was significant.

Of the sows that were present in the herd at weaning of their first litter, there was a significant treatment effect on the cause of sow retention to subsequent re-mating (Table 10). There were fewer mortalities and locomotion culls in the Omega-3 supplemented sows, but a higher proportion of anoestrus sows compared to the Control sows. There were similar losses post-mating in parity 1 for reproductive reasons in the Omega-3 sows (13.3%) compared to the Controls (13.0%). Although there were higher losses due to anoestrus, the net effect of supplementation on retention remained positive when Omega 3 diets were fed.

The retention of sows was improved in the Omega-3 fed gilts and sows with the treatment difference significant at 85% confidence by the second litter (parity 2) farrowing (Table 11; Figure 7). When the data was analyzed for cause of sow removal, there were significantly fewer sows removed ( $P < 0.01$ ) from the Omega-3 treatment group due to physical causes, including sudden death, downer sow, body condition, feet and leg culls and destructions (Figure 8). The effect of dietary treatment within category was not statistical, but feet and leg culls and destruction for lameness (downer sow syndrome) were lower in the Omega-3 sows (59/229 removals) compared to Control sows (85/261), and supports the observation seen during the post-weaning period alone (see Table 10).

Table 9. Reproductive performance (mean±SE) in first and second parity of sows fed diets supplemented with omega-3 fatty acids from 3g fish oil/kg over two parities.

	Control	Omega-3	P value
No. gilts allocated	984	974	
Gestation parity 0 (d)	115.8±0.05	115.7±0.05	0.419
Gilt farrow rate (%)	90.8%	91.6%	x <sup>2</sup> 0.2, 0.656
<i>Parity 1</i>			
Live born	10.8±0.09	10.8±0.09	0.698
Still birth (% of total born)	6.1±0.33	6.6±0.33	0.310
Total born	11.8±0.09	11.7±0.09	0.694
Wean to mate interval <sup>1</sup> (d)	25.9±0.22	25.2±0.22	0.017
Proportion re-bred within 28 days post-weaning	75.4%	80.1%	x <sup>2</sup> 5.99, 0.014
Gestation parity 1(d)	116.0±0.05	116.1±0.05	0.375
Parity 1 farrow rate	84.0%	84.4%	x <sup>2</sup> 0.04, 0.843
<i>Parity 2</i>			
Live born	12.1±0.11	12.2±0.10	0.677
Still birth (% of total born)	7.2±0.38	7.2±0.38	0.918
Total born	13.1±0.11	13.2±0.11	0.593
Cumulative live born Parity 1 and 2	18.6±0.27	19.1±0.27	0.158

<sup>1</sup>Time block a significant main effect included as a random factor in GLM ANOVA model.

Table 10. The breakdown of reasons for parity 1 sow removal post-weaning to re-mating for sows fed supplemented and un-supplemented Omega-3 diets with fish oil.

	Sows weaned	Re-mated	Mortality	Culled anoestrus	Culled locomotion	Sum physical	Other reasons
Control	883	806	9 <sup>x</sup>	16 <sup>x</sup>	46 <sup>a</sup>	55 <sup>a</sup>	6
Omega-3	881	820	4 <sup>y</sup>	27 <sup>y</sup>	25 <sup>b</sup>	29 <sup>b</sup>	5
Chi square value	0.17	1.49	2.78	2.91	6.42	8.39	0.09
P value	0.681	0.223	0.095	0.088	0.011	0.004	0.765

<sup>ab</sup>Within removal reason treatment differences were statistically significant P<0.05 or <sup>xy</sup>P<0.01.

Table 11. The number of sows present within the breeding herd when fed diets containing omega-3 fatty acids from fish oil continuously during gestation and lactation and subsequent gestation compared to sows fed un-supplemented diets.

	Gilt mating	1 <sup>st</sup> litter farrow	1 <sup>st</sup> litter weaned	Parity 1 mating	2 <sup>nd</sup> litter farrowed
Control	984	894	883	806	714
Omega-3	974	892	881	820	738
Chi square value		0.2	0.17	1.49	2.21
P value		0.656	0.681	0.223	0.137

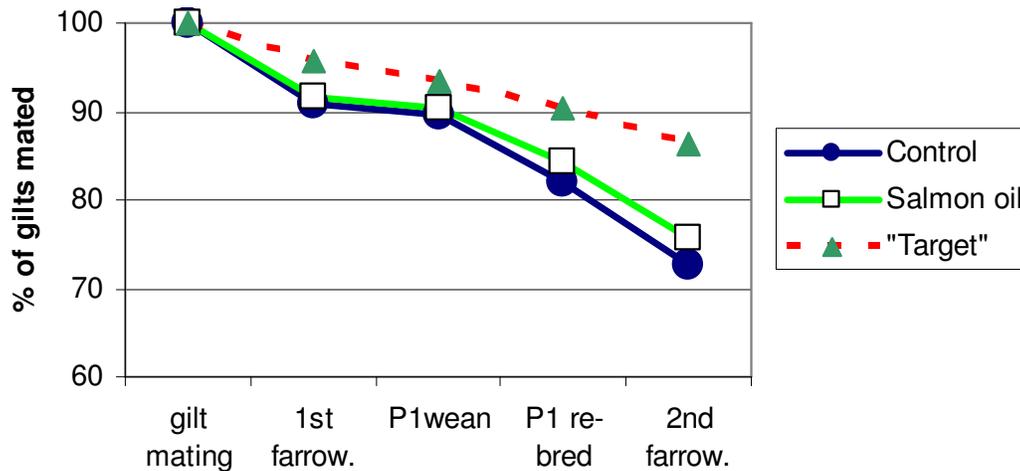


Figure 7. Sow retention from gilt mating within the breeding herd when fed diets supplemented with 3 g/kg of omega-3 fatty acids from fish oil continuously over the first two parities (from Table 8) compared to a target suggested from Sow Productive Lifetime Conference, Nashville Indiana, 2007).

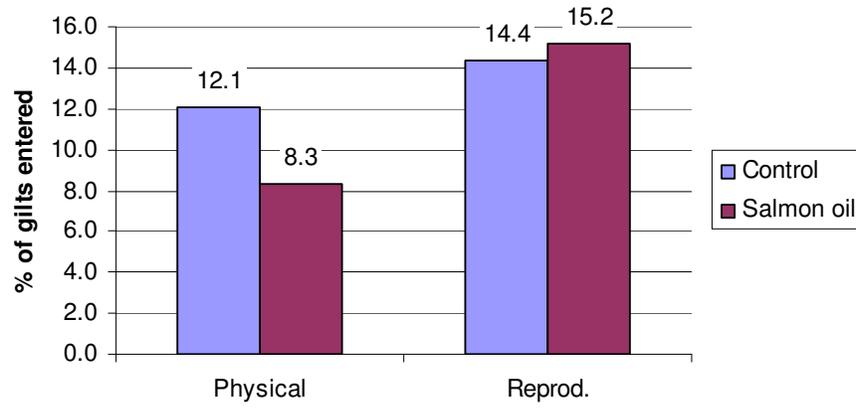


Figure 8. Sow removal over parity 0 (gilt gestation) and parity 1 (to point of farrowing 2<sup>nd</sup> litter) due to physical or reproductive causes between omega-3 supplemented and un-supplemented feeding treatments.

## Discussion

We found there was no significant effect of long-chain omega-3 fatty acid supplementation with fish oil in gilts prior to mating on oestrus, litter size born, or pregnancy rate. The gilt response to fish oil supplementation is supported by the findings of Estienne *et al* (2006), who also reported no effect of long-chain omega-3 PUFA supplementation embryo number or embryo survival, but is in contrast to the finding reported by Spencer *et al* (2004). Both of these studies used a commercial protected form of PUFA's from fish oil (Fertilium™) in a corn-soybean meal based diet and Estienne *et al* (2006) reported that 1% supplementation resulted in a dietary EPA and DHA concentration of 0.6 g/100 g fatty acids. By comparison, this was considerably higher than the dietary EPA and DHA levels analyzed in our supplemented diet using 3 g/kg (0.3%) of liquid fish oil (0.27 and 0.35 g/100 g fatty acids, respectively). Plasma levels of fatty acids were not reported by Estienne *et al* or Spencer *et al*. The level of supplementation, or the ratio of omega 6 to omega-3 fatty acids, may be an important factor in the reproductive response in gilts (Estienne *et al*. 2006). The plasma fatty acid profile analysis from pubertal gilts showed that dietary supplementation is highly effective at increasing the concentration of the long-chain omega-3 fatty acids EPA (C20:5) and DHA (C22:6). We found that the uptake as free fatty acids in plasma is evident after only three days from diet introduction, and the concentration continued to increase up to day 21. Following on from the results from Experiment 1, we increased the supplementation level in Experiment 2 from 3 to 10 g fish oil/kg. This provided a higher level of dietary EPA (0.68 vs 1.65 g/100 g fatty acids) and DHA (1.74 vs 2.93 g/100 g fatty acid) by mating after six weeks supplementation when compared to supplementation at 3 g/kg fish oil. These plasma results confirm that dietary supplementation at low to moderate levels has substantial effects on the circulating levels in the body and also confirm previous findings that supplementation with omega 3 EPA and DHA reduces the plasma level of the omega 6 acid, ARA (Smits *et al*. 2007).

The analysis of the fatty acid profile in follicular fluid showed that at 10g/kg supplementation, the provision of 10g fish oil /kg significantly changed the concentration of various fatty acids in the follicular fluid surrounding the developing oocyte in the ovary, most notably the total amount of n-3 fatty acids and the long- chain fatty acids, EPA and DHA. These results indicate that the oocytes are developing in an enriched environment, similar to the profile reported for plasma. Before an oocyte is fertilized, it must undergo maturation to the metaphase II stage of meiosis and we were able to study this using an in-vitro maturation culture system. A small experiment in which oocytes were recovered from the ovary and matured in vitro, revealed little difference between control and omega-3 supplemented gilts in the number of follicles present on the ovary. However, when these oocytes were examined following in vitro culture, more oocytes from the larger follicles of omega-3 supplemented gilts were mature, relative to those from control animals. Improved oocyte maturation could potentially lead to greater fertilization rates

and the production of more embryos. Such oocyte and follicular characteristics have not been examined before, and indicate that there may be beneficial effects of omega-3 supplementation on the follicular environment and the ability of the oocyte to mature, which was performed in Experiment 3b in mature sow oocytes. Experiment 2b in gilts did not include a dietary treatment level at 3 g/kg, and the outcome in Experiment 2a showed that embryo survival was not linearly related to increasing fish oil supplementation at 10 g/kg, but was actually maximized numerically at 3 g/kg. Although we recorded ovulation rate as the total number of corpora lutea, the size of the follicle prior to ovulation may be an important indicator of fertility that is difficult to quantify unless each corpus luteum is dissected and weighed individually.

#### *Responses on reproductive performance, ovulation rate and embryo survival*

There was a significant increase in the number of days taken to mating ( $P < 0.05$ ) which was associated with a tendency for a longer onset of puberty in those gilts that were fed the supplemented omega-3 diet from 24 weeks of age. Experiments 1 and 2 showed that there was no response to gilt supplementation in conception or farrowing rates, embryo survival or litter size at day 25 of gestation or at term. In Experiment 2 when gilts were supplemented with 3 g of fish oil/kg, ovulation rate was also unaffected by dietary treatment and we did not observe any increase in ovulation rate, embryo number or embryo survival by feeding a higher level of fish oil (10 g/kg). Progesterone is critical for the successful implantation (Ziecik *et al.* 2006) and the support of the litter after implantation (Jindal *et al.* 1997). The tendency for an improved embryo survival may have been caused by either a better quality embryo during peri-implantation or more functional corpora lutea producing progesterone and lowering antiluteolytic prostaglandin  $\text{PGF}_{2\alpha}$ . The results from experiment 2 support the findings in gilts fed supplemented diets up to mating in experiment 1 and also those reported by Estienne *et al.* (2006) who did not observe any effect of supplementation on ovulation rate, embryo number or embryo survival in gilts. In their experiment, Estienne *et al.* used altronogest (Regumate) to synchronize ovulation which may have influenced their outcomes. Altronogest artificially increases progesterone and ovulation rates in treated gilts have been reported to be higher than non-synchronized gilts (Martinat-Botte *et al.* 1995; Soede *et al.* 2007).

Increasing the level of supplementation to 10g fish oil/kg of feed did not improve the response when litter size was assessed at 25 days of gestation. The gilt embryo survival and litter size was high in these experiments, and nutritional limitations due to inadequate omega 3 fatty acids on embryo survival may not have limited litter size in gilts. The experimental data produced from these experiments provide valuable information as to the potential fecundity of commercial genotypes in Australia. The embryo survival in these naturally cycling gilts was consistent with, if not higher to those reported for gilts overseas (Almeida *et al.* 2000; Estienne *et al.* 2006; Foxcroft *et al.* 2006). There was a high level of fertility in the commercial gilt studies in Experiment 1

and 2, and any possible improvements through omega 3 supplementation may not have been possible due to other limitations, such as placental size. Variability in embryo number and litter size is quite large and these results may explain why some commercial outcomes have not been consistent.

As there appeared to be no beneficial response to dietary omega 3 supplementation in gilts at either 3 or 10 g fish oil/kg, we decided to focus the research direction in older sows. Earlier studies using fish oil at 3 g/kg at Rivalea Australia showed that litter size born and born alive in multiparous sows fed supplemented diets during the previous lactation was increased by +1.0 piglet (Smits, unpublished). In this earlier study, the population litter size was commercially low (9.3 Controls v 10.3 Omega 3). The results from Experiment 3a support this outcome although a further large scale study in supplemented sows during lactation in sows which are performing at a higher level is needed for a proper economic cost-benefit. In contrast to the gilt slaughter study, there was no reduction in ovulation rate observed in the mature sows with supplementation at 3 g fish oil/kg. The physiological mechanism involved responsible for the observed increase in embryo survival in Experiment 3 is still to be determined. However, the results of Experiment 3b indicate that dietary supplementation may be exerting an effect through changes to the follicle environment surrounding the pre-ovulatory follicle and consequently the ability of the fertilized oocyte to develop.

The role of omega-3 long chain PUFA's in reproductive metabolism is thought to be through prostaglandin synthesis via altered eicosanoid metabolism, changes to progesterone and/or folliculogenesis (Staples *et al.* 1998). In fishmeal supplemented rations fed to dairy cattle, Mattos *et al.* (2002) and Thatcher *et al.* (1997) reported that  $\text{PGF}_{2\alpha}$  was reduced during pregnancy and this antiluteolytic mechanism may explain the reported benefits to pregnancy rates in cattle fed supplemented diets (Staples *et al.* 1998). Conversely, Petit *et al.* (2002) reported an increase in  $\text{PGF}_{2\alpha}$  in cows when diets were compared at a very low omega 6:omega-3 ratio (<2). There may be a different physiological response depending on the amount of long-chain omega-3 PUFA's supplied. In both gilts and sows we found that supplementary dietary omega 3 from fish oil increased EPA and DHA in the follicular fluid, whilst there was a significantly lower concentration of the omega 6 acid, ARA. The negative relationship between omega 3 supplementation and ARA has been reported previously in plasma of pubertal gilts (Smits *et al.* 2007). ARA is the preferred precursor to the eicosanoid pathway that produces the inflammatory response in the body through leukotrienes, thromboxanes and prostaglandins (Caughey *et al.* 2005). Although little is understood as to how omega 3 fatty acids may influence embryo survival, it may be mitigated through effects on the eicosanoid pathway and the production of prostaglandins and possibly progesterone. The omega 6 acid, ARA can be substituted for the omega 3 acid, EPA, which could alter the sows' physiological responses (James *et al.* 2000). Evidence for this is from Experiment 3b where dietary differences were evident in gene activation of COX-2, prostaglandin and progesterone

receptors, all of which were decreased with in follicular granulosa cells from omega 3 supplemented sows. Altered prostaglandin activity may be an explanation for the observed delay to the onset of oestrus as seen in Experiment 1 and the tendency for a higher rate of anoestrus weaned parity 1 sows recorded in Experiment 4. If circulating  $\text{PGF}_{2\alpha}$  is reduced, the hormonal cues for the onset of ovulation following the follicular phase of the oestrus cycle may have been delayed.

Embryo survival after fertilization is highly dependent on the signals for maternal recognition of pregnancy (Ashworth 1991; Love *et al.* 1993; Roberts *et al.* 1993). Before day 9, there is a high rate of survival from fertilized ova and it is generally understood that most of the early embryo loss occurs around period of maternal recognition and implantation (Roberts *et al.* 1993). Maternal recognition occurs in pigs between days 10-12 of pregnancy when the young embryo hatched from the zona pellucida between 6-7 days of age begin to migrate as blastocysts within the uterine horns and elongate (Ashworth 1991; Geisert and Yelich 1997). Oestrogen production from embryos is still regarded as a major determinant of embryo survival through its effects on elongation of the embryo and uterine receptiveness. Related to conceptus-derived oestrogen is the production of progesterone from the corpora lutea of the ovary. In non-pregnant cyclic animals, progesterone production from the CL is inhibited by the synthesis of  $\text{PGF}_{2\alpha}$  from the uterus, causing regression of the CL by day 15-16, thereby allowing the next ovulation in the oestrus cycle to occur. During pregnancy, oestrogen from the conceptus causes a re-direction of  $\text{PGF}_{2\alpha}$  from the blood supply to the uterine lumen (inside the uterine horns) where it is inactivated. Without  $\text{PGF}_{2\alpha}$  in the blood supply, the CL can continue to function and produce progesterone which maintains the pregnancy and growth of the conceptus.

The mechanism by which omega 3 fatty acids affects embryo survival remains unsolved from this project. However, the results from Experiment 3a in mature sows showed that omega 3 fatty acids from fish oil significantly affects survival and consequently litter size by three weeks of pregnancy after implantation has occurred. Previous research has investigated the effects of high feeding levels or energy levels on progesterone-mediated effects on embryo survival (Jindal *et al.* 1996; Jindal *et al.* 1997). Grandhi (1988) reported that supplemental tallow in diets from puberty to mating reduced embryo survival in gilts. In general practice, feed intakes and low daily energy intakes post-mating for three weeks are adopted to minimize any risk of adverse effects on embryo survival. Our experimental results in sows showed that the nature of the energy source from fat has an effect on embryo survival. Both the treatment diets contained the same level of energy and were fed at the same daily level during early pregnancy. The low level of supplementation using 3 g fish oil/kg prior to mating in any of our experiments did not appear to have any discernable energy effect with no differences in pubertal weight gain or fat gain in gilts (Experiment 1 and 2), nor on lactation performance, feed intake or sow weight loss (Experiment 3). The response of embryo survival therefore appears to be

due to the specific nature of the omega 3 fatty acid profile, and not due to an energy or feed intake response.

Oocyte and embryo development were examined *in vitro*, following supplementation with 3g fish oil/kg in sows. Although the number of developing follicles did not differ significantly between the control and omega-3 supplemented diet, embryo development to the blastocyst stage was significantly greater for the supplemented sows. This is the most advanced stage examined *in vitro* prior to embryo transfer, and is the stage at which elongation and implantation begins to occur. This beneficial effect is in contrast to impaired embryo development reported in the mouse (Wakefield et al 2008), however a relatively large concentration of fish oil was supplied in that study which makes comparisons between studies difficult (10 x dietary EPA and 2 x dietary DHA).

To begin to understand the intrinsic mechanisms which may contribute to the improvement in embryo development, we examined embryo metabolism of *in-vitro* fertilized sow oocytes in experiment 3b. Dietary supplementation at 3 g/kg was shown to increase the concentration of EPA and DHA in follicular fluid, and tended to increase the proportion of medium to large follicles on the ovary, which supported the earlier findings in gilts fed at 10 g fish oil/kg. Perturbations in embryo metabolism have been associated with reduced embryo development as a consequence of diet in a number of species (Mitchell et al 2009 *in press*). At the blastocyst stage, glucose is the primary carbohydrate source and it undergoes oxidative metabolism (requiring the consumption of oxygen) for the formation of ATP for energy for the growing embryo. Although embryo respiration rate tended to be greater for early embryos (single-cell stage) generated from oocytes from omega-3 fed sows, the rate of glycolysis was significantly reduced for blastocysts. This may be reflective of more efficient glucose metabolism and utilization at the later stage of development, thus more detailed analysis of embryo metabolism is warranted to further unravel the mechanism of improved embryo development. The cell culture studies also revealed some interesting findings in the dietary effects on gene activation. Gene activation of the progesterone and prostaglandin receptors in the follicle tended to be down-regulated in the sows fed 3 g/kg fish oil. It is not known how this may affect apparent dietary effects on embryo survival, but a possible hypothesis is that luteolytic  $\text{PGF}_{2\alpha}$  may have a decreased negative impact on the ovarian support of the blastocyst and embryo around the time of implantation.

The impact on litter size at full term was not studied in Experiment 3, however the positive response on embryo number by early gestation supports previous experiments in multiparous sows which showed that subsequent litter size born live and total born is increased when fish oil is supplemented to diets offered *ad libitum* during lactation (Smits, unpublished).

### *Responses on lactation performance*

There was no effect of dietary omega 3 acids at 3 g/kg on litter weight gain, sow feed intake or sow weight loss in Experiment 3. This is consistent with the findings reported by Philpotts and Henman (2007) who also used the same level and ingredient source of fish oil. We saw no evidence of any inappetance, nor did it promote feed intake when offered *ad libitum* during lactation. In the early studies where Palmer *et al.* (1970) used fish meal as a supply of protein, it was thought that the response may have been due to energy changes or protein supply during lactation (Reese 2003). Using only small quantities of supplementation, and through tallow substitution by volume, we were effective in creating iso-energetic diets. There was no indication that the dietary change in PUFA profile of omega 6 to omega 3's or the predicted changes expected in the plasma (not recorded in Experiment 3a) on EPA, DHA and lower ARA had any effect on lactation performance, piglet birth weight or development. Philpotts and Henman (2007) concluded that the level of supplementation in lactation may have been too low for developmental effects on the neonate, such as reported by Rooke *et al.* (Rooke *et al.* 2001a; 2001c) and Leskanich and Noble (1999).

### *Responses on sow removal and longevity in young parities*

The supplementation of long-chain omega-3 fatty acids in sow diets was shown to significantly improve post-weaning resumption of oestrus and sow longevity in the commercial evaluation of Experiment 4. The response observed in improving post-weaning oestrus was small, but given the skip-a-heat mating practice, the possibility of a larger response without this resting period after weaning may be expected. There was a greater retention of parity 1 sows between weaning to re-mating during the holding period mainly due to fewer losses for lameness and mortality. The results suggest that the magnitude of response to omega 3 supplementation on physical health may only apply to the sow as she gets older, or improved health with sustained intake of omega 3 fats. There was no difference in retention rate of mated gilts during gestation or first lactation.

There was a higher incidence of anoestrus sows in the Omega-3 sows (3.1% weaned sows) after parity 1 weaning compared to un-supplemented sows (1.8% weaned sows). In Experiment 1 using 3 g/kg fish oil fed to gilts prior to mating, we observed no difference in gilt wastage due to anoestrus culling. However, we did record a significant increase in mating age in gilts when fed supplemented diets for six weeks prior to mating. In Experiment 2, we also observed that there were fewer gilts that cycled when on fish supplemented at 3 g/kg, though this difference (71.4% vs 79.3%) was not significant. In contrast, in mature sows used in Experiment 3, there was no indication of a delay in oestrus, or a decrease in ovulation rate or conception rate in the supplemented sows. Published studies have not reported on adverse effects on oestrus onset in pigs, and it is interesting as to why this may have occurred. There may be an effect of omega-3 fatty long-chain PUFA's on the hormonal control of oestrus onset via the hypothalamic-pituitary-

ovarian axis that still needs to be determined, and consequently feeding regimens adjusted to reduce post-weaning anoestrus. Nevertheless, the overall outcome was for a higher proportion of sows supplemented with omega-3 PUFA's to be retained in the herd up to second parity.

Other benefits in reproduction performance were not observed in this study, although we recorded an improvement of 0.5 live born over the first two parities at  $P < 0.10$ . At 90 % confidence for statistical testing at  $P < 0.05$  for a two-tailed test of significance, we would have required nearly 6,000 sows per treatment to have detected an improvement of 2.6% in cumulative litter size given the standard deviation of 8.36. Nevertheless, half a pig difference on average, or 534 piglets born live per 1000 gilts mated over the first two parities is commercially important at a value of \$40 a piglet. In terms of improved retention of breeding sows due to reproductive causes, there was no difference in the sow retention rate between supplemented and un-supplemented sows. The fertility of gilts and parity 1 sows was notably high in the data set, so improvements through supplementation may be constrained by genetic potential, rather than any physiological limitations. In Experiment 3, we recorded a significantly higher embryo survival and litter size by early pregnancy. The difference in outcomes between experiments may be due to the mature sows used in Experiment 3 being more compromised than the younger sows used in Experiment 4.

Our results provide new information on the benefits of omega-3 PUFA supplementation from fish oil on sow longevity. As hypothesized from human and companion animal nutritional studies and commercial practice, the proportion of sows removed from the breeding herd due to physical causes including lameness, sudden death and body composition, was lowered by 31%. As with other species, it is likely that the change in long-chain PUFA from arachidonic acid, ARA (n-6) to the less inflammatory eicosapentanoic acid, EPA (n-3), reduced arthritis and joint tenderness. When the data was further broken down to removal for lameness only, the effect of long-chain omega-3 supplementation was clearly evident.

Sow wastage due to physical removal reasons is increasing in the commercial herd. In a sow survey conducted in 2003 at Corowa across all parities, physical removals accounted for 12% of sow culls (Hughes and Smits 2002). This was in agreement with other published studies in other countries (Hughes and Varley 2003). Although reproductive failure or low fertility is the primary cause of low sow longevity within modern commercial breeding herds, sow health and culling for feet and legs is becoming an increasing proportion of premature sow culling or destruction. A reduction in lameness culling and destruction recorded in this study by supplementing diets with low inclusion rates of fish oil provides a non-antibiotic and non-steroid solution. The data indicated that as age increases, or the duration of feeding increased, the effects on sow retention became more evident.

In conclusion, the project has evaluated the response in fertility and longevity to dietary supplementation of fish oil. In the pubertal gilt and during early gilt pregnancy, there was no evidence of a significant beneficial effect in reproductive performance. In the multiparous sows, supplementation with low levels of fish oil at 3 g/kg resulted in significant increases in embryo survival and embryo litter size that could correspond to higher multiparous litter size and farrowing rates. Sow longevity also was improved with supplementation during gestation and lactation, particularly through a reduction in mortality and lameness. The use of higher omega 3 supplementation using fish oil provides an alternative to antibiotic treatment for possible inflammatory causes of ill-health and culling in multiparous sows.

## Application of Research

*Application of the research findings in the commercial world.*

Fish oil for commercial feed formulation now has to be further processed due to a change in importation requirement from AQIS to protect Australia's aquaculture industry from possible disease from contaminated ingredients. As a result, raw fish oil can no longer be commercially imported, and this has increased the cost of fish oil as a supplement. The benefits in terms of fertility and sow health and lower replacement rate are most likely to occur in multiparous sows.

The cost of supplementing the diet with fish oil (\$3.65/kg) at 3 g/kg is 0.011 cents/kg.

	<u>Σ Feed/sow</u>	<u>Rate/kg</u>	<u>Cost/sow</u>
Lactation Parity 1 for 23 days	150 kg	3 g/kg	\$1.65/sow
Post-weaning P1 5 days	15 kg	6 g/kg	\$0.50/sow
<u>Parity 1 gestation</u>	<u>300 kg</u>	<u>6 g/kg</u>	<u>\$6.60/sow</u>
			\$8.75/sow per cycle

- Of 1000 sows, assume a retention of 40 sows per cycle  
Costing as a replacement @\$440/gilt \$17.60 per sow per cycle
  - Assume an additional 0.25 live piglets per multiparous sow  
from Parity 1+ @\$40 a head/piglet \$10.00 per sow per cycle
- Return* \$27.60/sow/cycle

*Return:cost ratio* 3.15:1

If the effects on litter size born through improved embryo survival are apparent in parity 3+ sows, then the ROI improves considerably.

- Assume an additional 1.0 live pigs per parity for 50% of sows over the replacement rate

\$17.60 per sow  
+ \$20/sow per parity  
*Return* \$37.60/sow/parity

*Return:cost ratio* 4.3:1

### *Opportunities uncovered by the research*

A commercial field study in multiparous sows to confirm the effects of experiment 4 on early pregnancy litter size is warranted under the base funding arrangement. A commercial evaluation on different sites would also allow individuals to determine their own economic assessment based on their individual herd performances.

The research in gilts also demonstrated that there is likely to be an optimum amount of supplementation based on omega 3:omega 6 fatty acid ratios. This still needs to be determined for the maximum profitable outcome and biological response.

### Commercialization/Adoption Strategies

- Potential benefits to cost of production - at 3:1 the economics justify its routine use.
- Ease of adoption by producers - Dietary formulations are easily adopted and commercial sources of fish oil are readily available. The use of un-processed and non-commercial fish oil sources are risky due to fatty acid profile imbalances and potential oxidative effects on feed quality.
- Impact of the research - The research program has identified that not all sows will benefit from supplementation and has recommended a targeted approach to dietary alterations. It has also highlighted that providing high rate of supplementation do not result in a greater response, and it is likely that there is an optimal level of omega 3:omega 6 fatty acid ratio that still needs to be quantified.

## Conclusion

There was no significant increase in puberty attainment, pregnancy rate or litter size born when a diet supplemented with 3 g fish oil/kg was offered *ad libitum* to unmated gilts from either 24 or 27 weeks of age. Feeding duration only affected the time taken to breed, all other reproductive traits recorded as similar between short and long periods of supplementary feeding. Gilts fed the omega-3 diet from 24 weeks of age through to mating and early pregnancy tended to have a higher embryo survival but effects of litter size born were not found to be statistically different.

In contrast to the gilt, in older sows we observed an increase in fertility with 3 g/kg fish oil supplementation during lactation and up to mating. There was a significant increase in embryo survival and litter size by early pregnancy in mature sows which exhibited typically low levels of fecundity, despite more than adequate ovulation rates. From the commercial longevity study where omega 3 from fish oil was fed continuously from first mating through to second parity farrowing, there was a significant improvement in sow retention due to a reduction in mortality and physical causes of ill-health when compared to un-supplemented controls. An increase in post-weaning anoestrus in

supplemented sows warrants further investigation as does a field study to determine the magnitude of improved embryo survival in multiparous sows.

## Acknowledgements

Some of these experiments formed part of the post-graduate PhD degree of Mr Robert Smits under the supervision of Assoc. Prof. Mark Nottle (Department of Obstetrics and Gynaecology) and Dr Megan Mitchell (School of Paediatrics and Reproductive Health), Medical School, The University of Adelaide, and Dr Brian Luxford, Rivalea Australia Pty. Ltd. The authors also express sincere gratitude to technical and research staff at Rivalea Australia Corowa, the Pig and Poultry Production Institute, SARDI, Roseworthy, and the Medical School, The University of Adelaide.

## Limitations/Risks

- The use of higher amounts of fish oil supplementation has been shown in the gilt studies to be of no benefit to reproductive performance. There has been no quantification as to the optimal level of omega 3:omega 6 fatty acids in sow diets, so the recommendation can only be given for the level of supplementation used in this project (3 g fish oil/kg diet).
- Post-weaning anoestrus in weaned parity 1 sows was identified as a risk in our experiment. In older mature sows fed supplemented diets there was no evidence of abnormal ovarian development.
- Producers and nutritionists need to use high quality fish oil with consistent fatty acid profiles as some sources vary markedly in quality in terms of fatty acid profile, contaminants and oxidative properties.

## Recommendations

With a return of 3:1, it is recommended that an inclusion of low levels of omega 3 fatty acids from fish oil to achieve a daily intake of 18 g/day is economical given the assumptions outlined above. Producers should review the responses on their own herds for a proper cost-benefit which could depend on their current level of performance and contributory factors predisposing their herd to low fertility and/or sow culling.

## References

- Almeida FRCL, Kirkwood RN, Aherne FX, Foxcroft GR (2000) Consequences of different patterns of feed intake during the estrus cycle in gilts on subsequent fertility. *Journal of Animal Science* **78**, 1556-1563.
- Ashworth CJ (1991) Embryo Development. *Pig News and Information* **12**, 551-554.
- Azain MJ (2004) Role of fatty acids in adipocyte growth and development. *Journal of Animal Science* **82**, 916-924.
- Caughey GE, James MJ, Cleland LG (2005) Prostaglandins and leukotrienes. In 'Encyclopaedia of Human Nutrition'. (Eds B Caballero, L Allen, A Prentice) pp. 42-49. (Elsevier: Oxford, UK).
- Clowes EJ, Aherne FX, Foxcroft GR (1994) Effect of delayed breeding on the endocrinology and fecundity of sows. *Journal of Animal Science* **72**, 283-291.
- Enser MI (1984) The chemistry, biochemistry and nutritional importance of animal fats. In 'Fats in Animal Nutrition'. (Ed. J Wiseman) pp. 23-51. (Butterworths: London, UK).
- Estienne MJ, Harper AF, Estienne CE (2006) Effects of dietary supplementation with omega-3 polyunsaturated fatty acids on some reproductive characteristics in gilts. *Reproductive Biology* **6** (3), 231-241.
- Foxcroft GR, Dixon WT, Novak S, Putman CT, Town SC, Vinsky MDA (2006) The biological basis for prenatal programming of postnatal performance. *Journal Animal Science* **84** (E. Suppl.), E105-E112.
- Geisert RD, Yelich JV (1997) Regulation of conceptus development and attachment in pigs. *Journal of Reproduction and Fertility Supplement* **52**, 133-149.
- Grandhi RR (1988) Effect of nutritional flushing, supplemental fat and supplemental lysine from puberty to breeding and during early gestation on reproductive performance of gilts. *Canadian Journal of Animal Science* **68**, 941-651.
- Hughes PE, Smits RJ (2002) Breeding herd feeding strategies to optimise reproductive efficiency and reduce culling rates. Australian Pork Limited, Final Report No. 1611.
- Hughes PE, Varley MA (2003) Lifetime performance of the sow. In 'Perspectives in Pig Science'. Nottingham. (Eds J Wiseman, MA Varley, B Kemp) pp. 333-355. (Nottingham University Press).

James MJ, Gibson RA, Cleland LG (2000) Dietary polyunsaturated fatty acids and inflammatory mediator production. *American Journal of Clinical Nutrition* **71** (suppl.), 343S-348S.

Jindal R, Cosgrove JR, Aherne FX, Foxcroft GR (1996) Effect of nutrition on embryonal mortality in gilts: Association with progesterone. *Journal of Animal Science* **74**, 620-624.

Jindal R, Cosgrove JR, Foxcroft GR (1997) Progesterone mediates nutritionally induced effects on embryonic survival in gilts. *Journal of Animal Science* **75**, 1063-1070.

Korver DR, Klasing KC (1997) Dietary fish oil alters specific and inflammatory immune response in chicks. *Journal of Nutrition* **127**, 2039-2046.

Leskanich CO, Noble RC (1999) The comparative roles of polyunsaturated fatty acids in pig neonatal development. *British Journal of Nutrition* **81**, 87-106.

Love RJ, Evans G, Klupiec C (1993) Seasonal effects on fertility in gilts and sows. In 'Fourth International Conference on Pig Reproduction'. Missouri. (Eds GR Foxcroft, MG Hunter, C Doberska) pp. 191-206. (*Journal of Reproduction and Fertility*).

Martinat-Botte F, Bariteau F, Forgerit Y, Macar C, Poirier P, Terqui M (1995) Synchronization of oestrus in gilts with altronogest: effects on ovulation rate and foetal survival. *Animal Reproduction Science* **39**, 267-274.

Mattos R, Staples CR, Williams J, Amorocho A, McGuire MA, Thatcher WW (2002) Uterine, ovarian, and production responses of lactating dairy cows to increasing dietary concentrations of menhaden fish meal. *Journal of Dairy Science* **85** (4), 755-764.

Palmer WM, Teague HS, Grifo Jr AP (1970) Effect of whole fish meal on the reproductive performance of swine. *Journal of Animal Science* **31**, 535-539.

Perez-Rigau A, Lindemann MD, Kornegay ET, Harper AF, Watkins BA (1995) Role of dietary lipids on fetal tissue fatty acid composition and fetal survival in swine at 42 days of gestation. *Journal of Animal Science* **73**, 1372-1380.

Petit HV, Dewhurst RJ, Scollan ND, Proulx JG, Khalid M, Haresign W, Twagiramungu H, Mann GE (2002) Milk production and composition, ovarian function, and prostaglandin secretion of dairy cows fed omega-3 fats. *Journal of Dairy Science* **85**, 889-899.

Reese DE (2003) Omega-3 fatty acids and swine reproduction - a review. In '2003 Nebraska Swine Report'. Lincoln pp. 30-33. (University of Nebraska Cooperative Extension - EC-03-219-A).

Roberts RM, Xie S, Trout WE (1993) Embryo-uterine interactions in pigs during week 2 of pregnancy. *Journal of Reproduction and Fertility Supplement* **48**, 171-186.

Rooke JA, Sinclair AG, Edwards SA (2001a) Feeding tuna oil to the sow at different times during pregnancy has different effects on piglet long-chain polyunsaturated fatty acid composition at birth and subsequent growth. *British Journal of Nutrition* **86**, 21-30.

Rooke JA, Sinclair AG, Edwards SA, Cordoba R, Pkiyach S, Penny PC, Penny P, Finch AM, Horgan GW (2001b) The effect of feeding salmon oil to sows throughout pregnancy on pre-weaning mortality of piglets. *Animal Science* **73**, 489-500.

Rooke JA, Sinclair AG, Ewen M (2001c) Changes in piglet tissue composition at birth in response to increasing maternal intake of long-chain *n*-3 polyunsaturated fatty acids are non-linear. *British Journal of Nutrition* **86**, 461-470.

Smits RJ, Luxford BG, Cadogan DJ (2007) Dietary supplementation with fish oil increases long-chain omega-3 polyunsaturated fatty acids in pubertal gilts. In 'Manipulating Pig Production XI'. Brisbane. (Eds JE Paterson, JA Baker) p. 46. (Australasian Pig Science Association).

Soede NM, Bouwman EG, Langendijk P, van der Laan J, Kanora A, Kemp B (2007) Follicle development during luteal phase and altronogest treatment in pigs. *Reproduction Domestic Animals* **42**, 329-332.

Spencer JD, Wilson L, Webel SK, Moser RL, D.M. W (2004) Effect of feeding protected *n*-3 polyunsaturated fatty acids (Fertilium™) on litter size in gilts *Journal of Animal Science* **82** (Suppl. 1), 211 (abstract).

Staples CR, Burke JM, Thatcher WW (1998) Influence of supplemental fats on reproductive tissues and performance of lactating cows. *Journal of Dairy Science* **81**, 856-871.

Thatcher WW, Binelli M, Burke JM, Staples CR, Ambrose JD, Coelho S (1997) Antiluteolytic signals between conceptus and endometrium. *Theriogenology* **47**, 131-140.

Webel SK, Otto-Tice ER, Moser RL, Orr DE (2004) Effect of feeding duration of protected *n*-3 polyunsaturated fatty acids (Fertilium™) on litter size and embryo survival in sows *Journal of Animal Science* **82** (Suppl. 1), 212 (abstract).

Ziecik AJ, Blitek A, Kaczmarek MM, Waclawik A, Bogacki M (2006) Inhibition of luteolysis and embryo-uterine interactions during the peri-implantation period in pigs. *Journal of Reproduction and Fertility Supplement* **62**, 147-161.