The functional role and requirement for long-chain omega-3 polyunsaturated fatty acids in breeding gilts and sows

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Abstract

The potential for supplemented omega-3 polyunsaturated fatty acids (PUFA) to increase sow reproductive performance when supplied from isocaloric diets containing low levels (3 – 6 g/kg of diet) of fish oil as a partial replacement for tallow was investigated. In the first experiment, there was an increase of 1 piglet live born ($P < 0.05$) to sows at the subsequent parity fed a supplemented diet before farrowing and during lactation. In contrast, litter size was unaffected when gilts were fed a supplemented diet with fish oil during puberty and early pregnancy. Furthermore in gilts, increasing the duration or level of supplementation did not improve litter size or embryo survival, possibly due to their inherently high level of fertility (82% embryo survival). In subsequent experiments, the effect of omega-3 supplementation on reproduction was evaluated in older parity sows known to have an inherently lower level of fertility compared with gilts. In parity 4 – 7 sows fed a supplemented diet prefarrowing and during lactation continuing to mating, embryo survival at 23 d was increased (Omega-3 70% vs 61% in Controls; P =0.054), without affecting ovulation rate. Subsequent experiments examined the response when supplemented diets were fed either during lactation continuing to mating; or after mating and during early gestation; or across both periods from lactation through to early gestation. Litter size born was maximised in the subsequent parity in sows fed fish oil diets from lactation to early gestation for 28 d, with the response being greatest in higher parity sows (+0.7 live born; and +0.9 total born, $P < 0.05$). In the following experiment this increases was associated with a 19% increase in embryo survival with omega-3 supplementation (P = 0.061). There was no effect on live weight or backfat during lactation; litter weight gain; piglet wean weight; and sow intake when gilts or sows were fed supplemented diets. The increase in embryo survival and litter size consistently observed in the sow studies was associated with increases in the omega-3 PUFA eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) and were independent of energy intake or energy metabolism. Partially replacing tallow (wt/wt) with 3 – 6 kg levels of fish oil did not change plasma levels of the essential omega-6 fatty acids, linoleic acid (LIN) and arachidonic

acid (ARA). Using in-vitro cultures of granulosa cells it was demonstrated that progesterone production is increased with prostaglandin E_3 and there was evidence for PGE_3 to enhance the steroidogenic response to PGE_2. It is proposed that specific long-chain omega-3 fatty acids increases embryo survival in older sows due to improved oocyte quality and/or embryo development, possibly through synergistic activities of PGE_2 and PGE_3 on progesterone levels in the local ovarian-uterine circulation. Supplementation of diets with EPA and DHA from fish oil offers pig producers a nutritional approach to improve sow litter size in older parities thereby increasing longevity and lifetime performance.

Keywords: Omega-3 PUFA, sow fertility, embryo survival, longevity, prostaglandins, progesterone

Declaration

I declare that this thesis is my own work and contains no material that has been accepted for the award of any other degree or diploma in any university or other tertiary institution to Robert J. C. Smits, and, to the best of my knowledge and belief, this thesis contains no material previously published or written by another person or persons, except where due reference has been made in the text.

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Publications arising

R.J. Smits, B.G. Luxford, M. Mitchell and M.B. Nottle (2011). Sow litter size is increased in the subsequent parity when lactating sows are fed diets containing n-3 fatty acids from fish oil. Journal Animal Science 89: 2731-2738

R.J. Smits, B.G. Luxford, M. Mitchell and M.B. Nottle (2011). Sow fertility is improved by feeding diets supplemented with omega-3 fatty acids from fish oil during lactation through to early pregnancy. Journal of Animal Science. 89 (E-Supplement 2). Abstract 192, P.109

R.J. Smits, D.T. Armstrong, L. Ritter, M. Mitchell and M.B. Nottle (2010). Progesterone production from granulosa cells of sows is enhanced equally by omega-3 derived prostaglandin E_3 and omega-6 derived prostaglandin E_2. Reproduction, Fertility and Development 22 (Supplement). P. 134 (abstract 334)

Co-authored publications

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Research reports

R.J. Smits and M. Mitchell (2009). Evaluation of supplementing long-chain omega-3 fatty acids as a nutritional approach to increase productivity and longevity in gilts and sows. Final Research Report, 2F-102. Pork CRC, Willaston, SA.

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General Introduction

Productivity from sows relies on a combination of variables that ultimately determine the fertility and longevity within the breeding herd. These variables include genetics, health, nutrition, management and husbandry factors. Nutrition of the sow as a prepubertal gilt through to higher parities has a large influence on reproductive performance. As well as supplying the macronutrients of energy and protein, the nutritionist must also consider the supply of specific essential nutrients for optimum performance. Fatty acids in pigs are essential in several important metabolic pathways. Dietary fats provide a rich source of energy for maintenance and production, as well as specific fatty acids that form components of cell membranes and supply substrates for metabolism (Rossi et al., 2010). For the sow to produce a large number of offspring over several years of productive life, the dietary supply of certain fatty acids is essential.

Essential fatty acids currently recommended for pigs are the omega-6 (n-6) fatty acids linoleic (LIN, C18:2) and arachidonic acid (ARA, C20:4; Enser, 1984). These polyunsaturated fatty acids (PUFA) undergo elongation and further desaturation to form long-chain PUFA which are required as substrates for the production of prostaglandins and other eicosanoids that are used in the regulation of a number of physiological activities including growth and development, reproduction, the immune system and inflammatory response (Caughey et al., 2005). Deficiencies in the supply of essential PUFA in the diet affect growth and reproduction. Recently there has been interest in the functional role of PUFA other than the omega-6 fatty acids LIN and ARA in the breeding sow, such as the long-chain omega-3 (n-3) fatty acids eicosapentaenoic acid (EPA, C20:5) and docosahexaenoic acid (DHA, C22:6). These are currently not regarded as essential fatty acids, however evidence from other species suggests that increasing the supply of these omega-3 PUFA may provide a new nutritional approach to increase reproduction in the breeding herd (Staples et al., 1998). Fish products, including fish oil, contain a high level of the long-chain omega-3 PUFA, EPA and DHA due

to their food source being derived from algae. Livestock that have been fed diets containing fish oil or fish meal as a source of protein or energy source have been reported to have improved reproductive performance (Palmer et al., 1970; Mattos et al., 2002). The responses have not been consistent, however, and a full understanding of the mechanisms involved is needed (Wathes et al., 2007). Furthermore there have been relatively few studies in gilts and sows, the results of which have been equivocal. Variables between published studies such as parity, duration of supplementary feeding, synchronisation of oestrus and level of supplementation may have influenced the results of these studies. Unpublished industry experience using fish oil and fish meal in diets fed to pigs have also suggested beneficial effects, but again these results have been variable. The relationship(s) between long-chain omega-3 supplementation of the diet and the physiological processes responsible for ovulation, embryo survival and maintenance of pregnancy has not been investigated in pigs.

The objective of this study was to quantify the reproductive response in gilts and sows fed diets supplemented with long-chain omega-3 PUFA from fish oil. Secondly, the experiments were designed to examine the mechanism(s) that may be involved in this response so as to describe the functional role of long-chain omega-3 fatty acids on reproduction in the pig.

Chapter 1 Review of the literature

1.1 Introduction

Essential fatty acids are required by mammals due to the lack of the desaturase enzymes that introduce double bonds between C_9 and the terminal methyl group of the fatty acid chain. Fatty acids considered essential for pigs are the omega-6 (n-6) fatty acids, LIN, (C18:2) and ARA (C20:4). Fatty acids that are denoted polyunsaturated (PUFA) are those with more than one double bond within the carbon chain. PUFA have different physical properties and metabolic functions than saturated or monounsaturated fatty acids that are supplied in the diet at levels that provide energy for metabolism. Although they are needed in much smaller quantities, PUFA are converted to important metabolites that are used in the regulation of a number of physiological activities including growth and development, reproduction, immune and inflammatory responses. Deficiencies in the supply of essential PUFA in the diet affect growth, health and reproduction. The long-chain omega-3 PUFA, EPA and DHA, are currently not regarded as essential fatty acids, however evidence from other species suggests that increasing the supply of these omega-3 PUFA may provide a new nutritional approach to improve reproductive performance (Staples et al., 1998).

The aim of the review is twofold. Firstly, an outline of the metabolism of fatty acids in animals is reviewed. Secondly, the literature describing known roles of omega-3 PUFA on the reproductive performance in gilts and sows is reviewed as well as possible mechanisms by which omega-3 PUFA may affect reproductive performance.

1.2 Structure, synthesis and metabolism of long-chain polyunsaturated fatty acids

PUFA are required as structural components of phospholipids in mammalian cell membranes and are precursors to prostaglandins, thromboxanes, hydroperoxy and hydroxy fatty acid derivatives and leukotrienes (Smith et al., 1983). Linoleic acid, LIN (C18:2, n-6) and α-linolenic acid, ALA (C18:3, n-3) are the dietary precursors of omega-6 fatty acids and omega-3 fatty acids, respectively.

1.2.1 Nomenclature of fatty acids

Polyunsaturated fatty acids are fatty acids with more than one double bond and are classed differently to saturated fatty acids (no double bonds) and monounsaturated fatty acids (one double bond; Kohlmeier, 2003b). Fatty acids are classified according to hydrocarbon chain length and the number of double bonds they contain and the position of these double bonds (Stryer, 1981). For example, LIN has 18 carbon atoms with two double bonds (C18:2), whereas ALA has a hydrocarbon chain length of 18 carbons and contains three double bonds (C18:3). Omega-6 fatty acids have a double bond between the sixth (i.e n-6) and seventh carbon atoms from the methyl (CH_3) carbon atom. Figure 1.1 illustrates the molecular structure of LIN. The position of double bonds in the unsaturated fatty acid hydrocarbon chain is referred to as *cis* (*eg. cis*-Δ^9, Δ^{12} refers to a double bond between C_9 and C_{10} and C_{12} and C_{13} in LIN). The number, type and position of these double bonds influence the physical and metabolic properties of fatty acids. The *cis* double bonds categorise the fatty acids with hydrogen atoms aligned on the same side (Kohlmeier, 2003b) and these differ to the less frequently occurring *trans* double bonds that have hydrogen atoms opposite sides of the two adjoining carbon atoms.

The difference between omega-3 and omega-6 fatty acids is shown in Figure 1.2, which represents ALA, which has the same carbon length as LIN, but a different location of the *cis* double bond. Regardless of carbon chain length or the number of double bonds, all omega-3 fatty acids have a

double bond between the third (i.e n-3) and fourth carbon atoms from the methyl carbon atom. The omega-3 ALA has three double bonds located at *cis*- Δ^9, Δ^{12}, Δ^{15}.

Linoleic acid (C18:2 n-6)

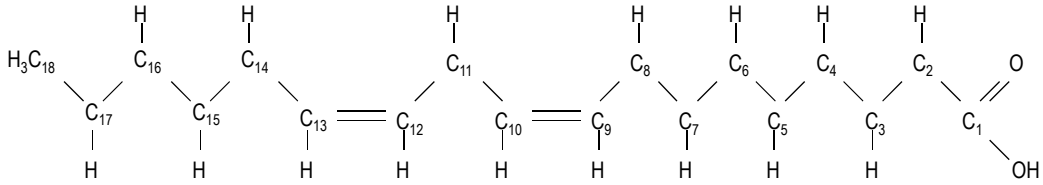

Figure 1.1 The molecular structure of linoleic acid (LIN), an 18 carbon chain omega-6 fatty acid, numbered from the carboxyl end to the distal methyl end and illustrating the cis-Δ9, Δ12 position of the cis double bonds

α-Linolenic acid (C18:3 n-3)

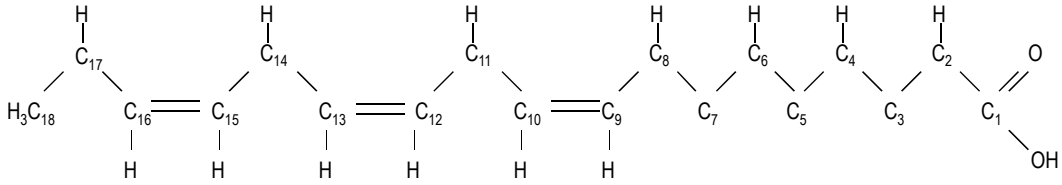

Figure 1.2 The molecular structure of α-linolenic acid (ALA), an 18 carbon chain omega-3 fatty acid, with a *cis*-Δ^9, Δ^{12}, Δ^{15} position

As well as the geometric isomers as *cis* and *trans*, fatty acids with an identical number of double bonds and carbon lengths can occur in multiple forms due to the location of the double bonds within the acyl chain. For example, the omega-3 α-linolenic acid (Figure 1.2) is different in its positional isomer configuration to γ-linolenic acid, which is the omega-6 form of linolenic acid. Enzymes that

metabolise fatty acids distinguish between both geometrical and positional isomers of fatty acids with a similar chain length. The metabolic outcomes from these different structures are specific and can have opposite physiological effects (Lichtenstein, 2005).

1.2.2 Fatty acid synthesis

Fatty acids are synthesised from acetyl CoA and bicarbonate. During this formation, a carboxyl (HCO_3^-) group from bicarbonate is attached at the start of the molecule (Figure 1.3). The synthesis of fatty acids occurs in the cytosol of liver, kidney, adipose tissue, brain, lungs and mammary glands in mammals (McDonald et al., 1988b) and the chloroplasts of photosynthetic plants and algae (Gurr, 1984).

From Diwan (2005)

Figure 1.3. Biosynthesis of malonyl CoA, the first step of fatty acid synthesis via the enzyme, acetyl CoA carboxylase

Elongation of the carbon chain of up to a chain length of C_{16} (palmitate) occurs by the sequential addition of two carbon units to the acyl chain from acetyl CoA in the fatty acid synthetase enzyme complex (Figure 1.4). Acetyl CoA is a product of the mitochondria and is transferred into the cytosol by the carrier molecule, citrate. Plants and mammals are able to synthesis fatty acid chain lengths of up to C_{16}, though the *de novo* synthesis in mammals of fatty acids is minor compared to that supplied from the diet (Kohlmeier, 2003b). Palmitate is the primary fatty acid produced from the fatty acid synthetase enzyme in the liver and adipose tissue.

From Diwan (2005)

Figure 1.4 Stoichiometry of malonyl- Co A elongation to palmitate (CH_3 $(CH_2)_{14}COO^-$) in the cytosol from the enzyme complex, fatty acid synthetase

Saturated palmitate (C_{16}) can then be elongated in the endoplasmic reticulum of the mitochondria by reverse oxidation pathways whereby acetyl CoA is systematically added to the carboxyl end of the fatty acid chain (Innis, 2003). There are four steps to fatty acid elongation: condensation, reduction, dehydration and reduction. As in synthesis, the additions of carbon atoms during the elongation reactions are supplied by malonyl-CoA via acetyl-CoA carboxylase (Innis, 2003).

1.2.3 Synthesis of polyunsaturated fatty acids

Mammals have a dietary requirement for PUFA because they do not possess the necessary enzymes (desaturases) capable of inserting a double bond between C_9 and the terminal methyl group of the acyl (carbon-hydrogen) chain (Enser, 1984; Herrera, 2002; Diwan, 2005). Linoleic acid and ALA are precursors for elongation and de-saturation reactions of PUFA that are of a chain length greater than C_{18}. These essential precursors contain the necessary double bonds at position C_{12} (LIN) and C_{15} (ALA) (refer to Figure 1.1 and 1.2). Hence these two PUFA must be supplied in the diet of animals and fish (Enser, 1984; McDonald et al., 1988b). Long-chain PUFA are those resulting from further de-saturation and elongation steps from LIN and ALA (Herrera, 2002). Arachidonic acid (C20:4 n-6) is a product of a three-step process of desaturation-elongation-desaturation of the omega-6 LIN with double bonds at positions C_5 and C_8 and carbon atoms added to the carboxyl end of the C18:2 molecule (Figure 1.5). The enzyme, Δ6-desaturase, is regarded as rate-limiting the conversion of LIN to the intermediatory γ linolenic acid (C18:3, n6) prior to the

synthesis of ARA and is known to be down-regulated by omega-3 PUFA (Garg et al., 1990). The omega-3 fatty acid, α-linolenic acid, is metabolised to form eicosapentaenoic acid (EPA) which can be further metabolised to docosahexaenoic acid (DHA; Figure 1.6).

The formation of ARA and EPA is dependent on the Δ5 desaturase enzyme which is present in unicellular algae (Corraze, 2001) but absent in plant cells and animal cells (Innis, 2003). Hence long-chain polyunsaturated omega-6 fatty acids of C20:4 *n*-6 or higher and omega-3 fatty acids of C20:5 *n*-3 are present in meat, fish and eggs mainly through the dietary intake of algae or other sources that consume algae, such as fish. Long-chain PUFA (> 18 carbon length) are not able to be supplied in large quantities from grains, oil seeds or vegetables (Enser, 1984).

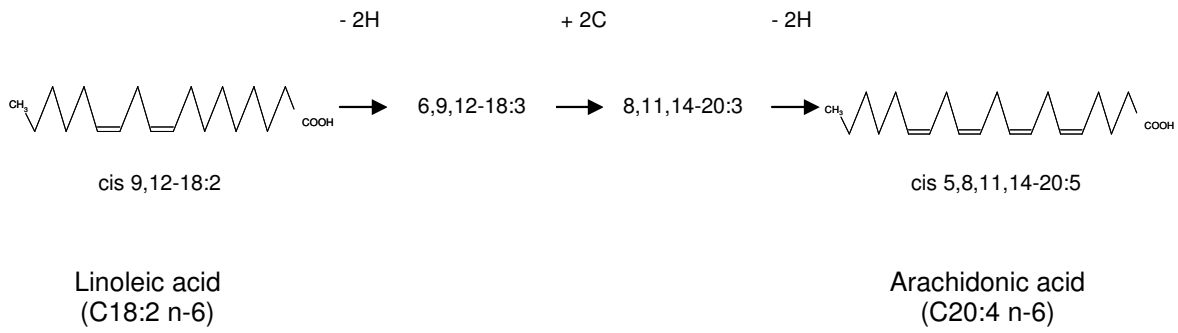

Figure 1.5 Metabolic pathway of the omega-6 fatty acid from LIN to ARA through desaturation-elongation-desaturation enzymatic reactions. From Enser (1984).

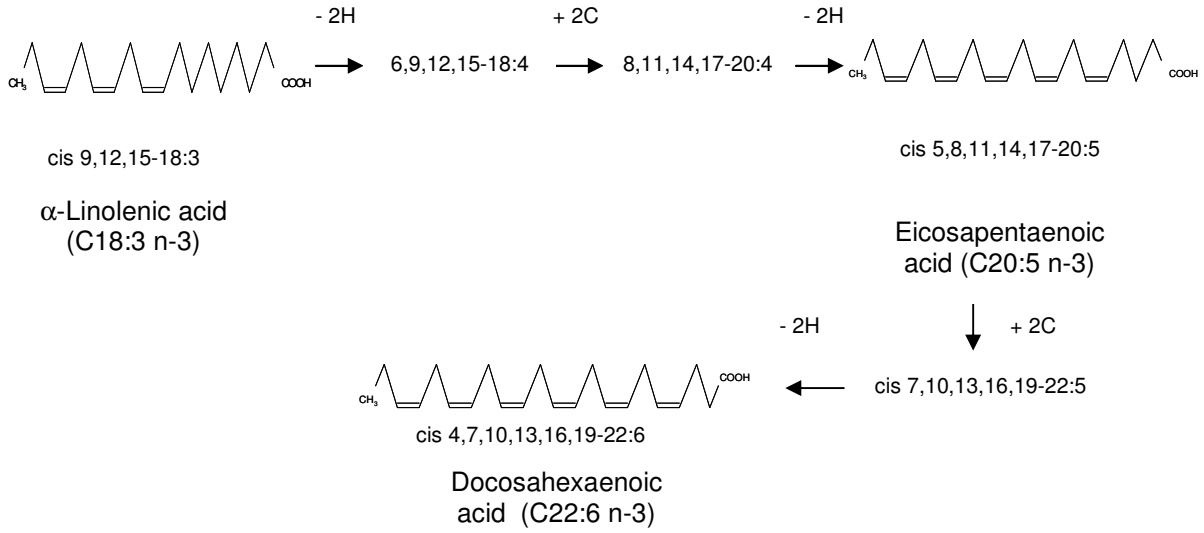

Figure 1.6 Metabolic pathway of the omega-3 fatty acid from ALA to EPA and DHA. From Enser (1984).

The synthesis of DHA is thought to be formed by elongation and shortened by β-oxidation. The intermediate, docosapentaenoic acid (DPA) 22:5 *n*-3, is elongated to 24:5 *n*-3, converted to 24:6 *n*-3 by Δ^6 desaturase, and shorted to 22:6 *n*-3 (DHA) by one cycle of peroxisomal β-oxidation (Innis, 2003; Kohlmeier, 2003a).

The polyunsaturated fatty acids ARA, EPA and DHA are precursors for the synthesis of hormonal-like substances called prostanoids (prostaglandins, prostacyclinin, and thromboxanes) and eicosanoids (leukotrienes and hydroperoxy and hydroxy fatty acid derivatives; Smith et al., 1983). The parent essential fatty acid structure as either n-3 or n-6 determines specific precursors from the omega-6 (ARA) or omega-3 (EPA) pathways. Each precursor fatty acid, in turn, generates eicosanoids and prostanoids with characteristic profiles (Kohlmeier, 2003b) and these can exert quite different effects on metabolism (Kohlmeier, 2003a; Wathes et al., 2007).

1.2.4 Bioconversion from short-chain essential fatty acids to long-chain PUFA

Although most mammals possess the necessary desaturase enzymes to synthesise long-chain PUFA from LIN, ALA and ARA acids supplied in the diet, the degree of biosynthesis may be inadequate for optimum metabolism and health. Azain (2004) reported from the literature that the conversion of ALA to EPA was only 15%, and that by feeding dietary sources of EPA from fish oil results in a much higher level of EPA in the body than observed when only ALA is supplied from the diet. In humans, *in vivo* biosynthesis of long-chain omega-3 PUFA occurs at a rate of 11 g ALA to 1 g long-chain EPA and DHA (Simopoulos, 2005). This efficiency is reduced considerably when there is an imbalance of n-6:n-3 PUFA, such as when a high level of LIN is supplied in the diet, or conversely a low level of ALA. Bioconversion is very limited in the young infant (Kohlmeier, 2003b) and neonatal pig (Leskanich and Noble, 1999) and in individuals with defective or absent peroxisomes (Kohlmeier, 2003b). Purvis et al. (1983) showed that although the foetal and young piglet was capable of bioconversion of LIN to higher chain PUFA, they could not conclude the rate was high enough to support the piglets' needs. Rooke et al. (2000) reported that increasing dietary supply of ALA during pregnancy in sows only resulted in minor increases (less than 10% of ALA) in EPA and DHA in the sow plasma and adipose tissue, which appears to be a lower conversion efficiency than reported in finisher pigs (Kloareg et al., 2007). Supplementation of diets with sources high in EPA and DHA substantially increases the concentration of these fatty acids in the plasma, adipose and tissues of animals (Perez-Rigau et al., 1995; Staples and Thatcher, 1999). There is evidence for a low rate of biosynthesis from ALA to long-chain omega-3 PUFA in sows. Perez-Rigau et al. (1995) reported that pregnant gilts fed a diet supplemented with menhaden fish oil had substantially higher levels of EPA and DHA in plasma than those fed a diet supplying twice as much ALA from soybean oil. Rooke et al. (2000) also showed that the level of long-chain fatty acids was increased substantially more when pregnant sows were offered diets with tuna oil (high EPA, DHA) compared to linseed oil (high ALA). It is for this reason that many researchers in human nutrition

and medicine now regard DHA as an essential fatty acid in the diet as it is needed in sufficient quantities for metabolism (Kohlmeier, 2003a).

1.2.5 Metabolism of omega-6 and omega-3 polyunsaturated fatty acids to eicosanoids

Long-chain PUFA are precursors for synthesising eicosanoids, the collective term to a family of molecules that exert a hormone-like effect on cells at a local level (Funk, 2001; Kohlmeier, 2003b; Hodgson and Mori, 2005). Eicosanoids have been found to have a wide range of functions including platelet aggregation, regulation of reproductive processes, inflammation (both pro- and anti-inflammatory responses), pain sensation, cardiovascular function, respiratory function, as well as being involved in the regulation of the gastrointestinal system and renal system (Caughey et al., 2005).

Prostaglandins are involved in the regulation of ovulation, fertilisation, implantation and parturition in breeding females (Caughey et al., 2005; Ziecik et al., 2006). The synthesis of prostaglandins and leukotrienes occurs via two pathways in mammals (Figure 1.7), firstly under the control of the cyclooxygenase pathway which produces the prostaglandins and thromboxanes, and secondly the lipoxygenase pathway that produces leukotrienes (Calder, 1996). The cyclooxygenase pathway involves the enzymatic isoforms, COX-1 and COX-2. Both are present in breeding gilts (Murphy et al., 2001), with up-regulation occurring around the time of embryo implantation (Geisert et al., 2005; Ziecik et al., 2006).

Arachidonic acid is the major precursor for eicosanoid metabolism in mammals and produces the 2-series prostaglandins including $PGE_{2\alpha}$ and PGE_2 (Funk, 2001; Figure 1.7). The omega-3 fatty acid EPA is interchangeable for ARA as the primary substrate in the formation of prostaglandins of the 3-series ($PGE_{3\alpha}$ and PGE_3; Figure 1.8). The 3-series eicosanoids are structurally different to the 2-series with different physiological properties, including a lower bioactivity (Calder, 1996; James et al., 2000; Caughey et al., 2005). As well as being an alternative precursor in the formation of

eicosanoids, dietary EPA and DHA from fish oil can reduce the concentration of ARA in plasma (Amusquivar et al., 2010) but only at high inclusion rates of fish oil in pigs (Smits et al., 2007). Dietary omega-3 DHA has been shown to inhibit the activity of Δ6 desaturase enzyme which synthesizes ARA (Garg et al., 1990; Raz et al., 1998).

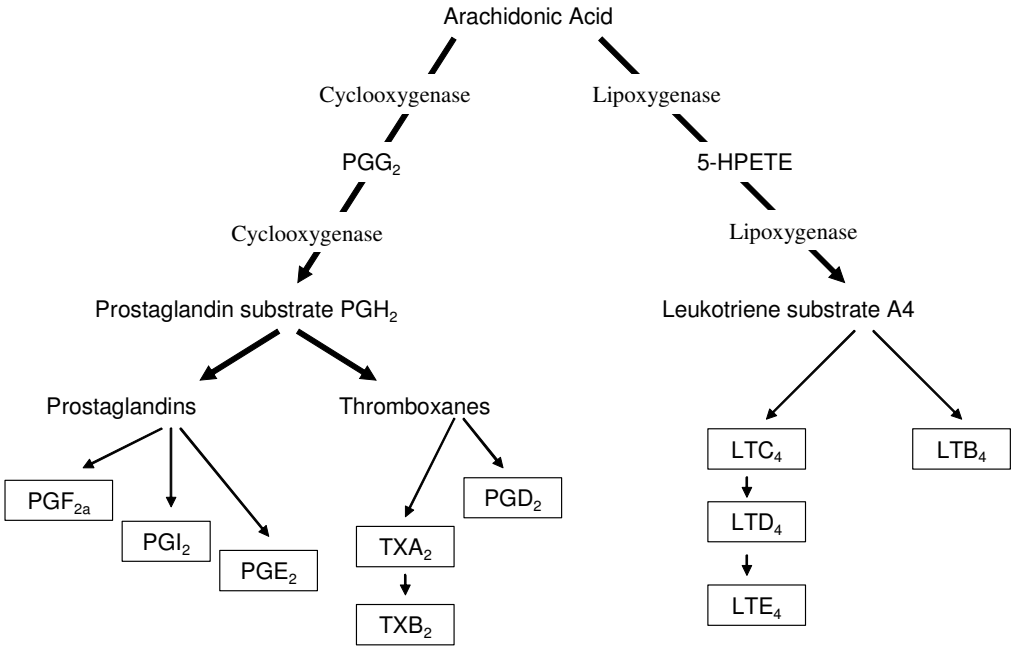

Figure 1.7 The metabolism of ARA (C20:4, n-6) to prostaglandins, thromboxanes and leukotrienes. From Caughey et al. (2005).

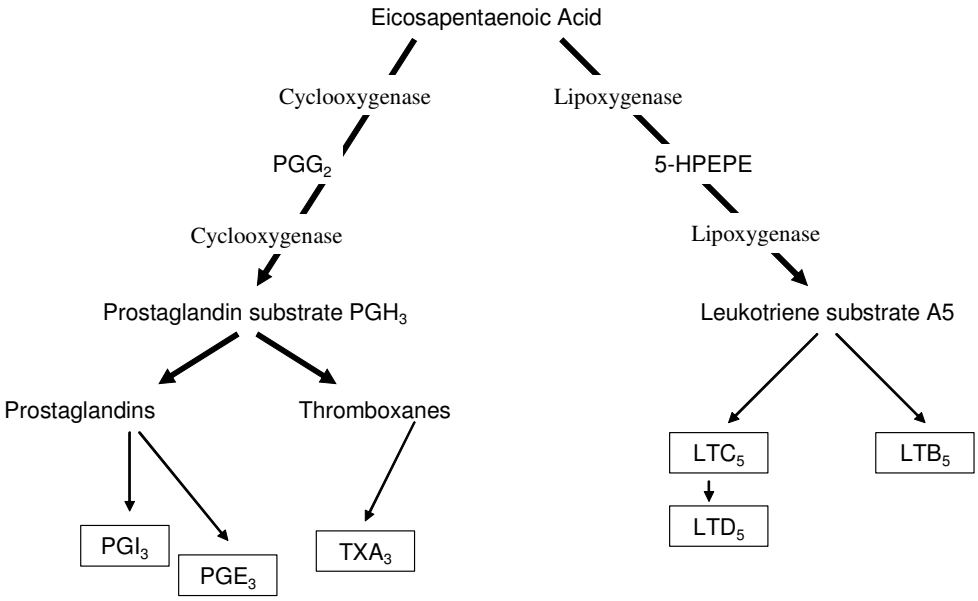

Figure 1.8 The metabolism of the PUFA, eicosapentaenoic acid (C20:5, n3) to prostaglandins, thromboxanes and leukotrienes. From Caughey et al. (2005).

1.3 Nutrient requirements for pigs

The nutrient requirements for pigs are documented through the international feeding standards published by the National Research Council in the US (NRC, 1998) and in Australia, The Standing Committee on Agriculture (SCA, 1987). These publications identified that pigs, as for other livestock, have an essential requirement for omega-6 PUFA as LIN and ARA. There is currently no recognition of a requirement for omega-3 fatty acids. Evidence of deficiency in essential fatty acids supply have been observed in extremely low fat diets fed to pigs and included dermatitis, necrosis of the skin and loss of hair (McDonald et al., 1988a; Close and Cole, 2000b) and poor growth and decreased viability of piglets (Cunnane, 1984).

1.3.1 Current nutrient requirements for polyunsaturated fatty acids in pig diets

The recommended allowance for LIN is 1.0 to 1.2 g/100g diet, although diets with as low as 0.1 g/100 g of the diet have shown normal growth from weaning to slaughter (NRC, 1998). Although recognising the importance of omega-3 essential fatty acids, the NRC (1998) concluded that practical pig diets containing cereal grains and protein meals will supply adequate levels for normal growth and metabolism and hence do not require special attention in dietary formulations. Perhaps the lack of overt symptoms of essential fatty acid deficiency, as suggested by Enser (1984), is the reason published feeding standards dismiss the dietary requirement of ALA and other long-chain PUFA of the omega-3 series in pigs.

Historically, there has been an acceptance in the literature for the nutrient requirement of pigs that diets containing sufficient quantities of LIN will also contain adequate quantities of ALA (Enser, 1984; NRC, 1998) and consequently adequate quantities of long-chain omega-3 PUFA will be available via *in vivo* biosynthesis in pigs. The rate of biosynthesis from LIN and ALA acid is low in animals (Azain, 2004; Simopoulos, 2005) and increased ALA through the diet is unlikely to result in an increase in EPA and DHA in placental tissues (Rooke et al., 2000; Rooke et al., 2001b). Thus in pigs, long-chain PUFA including ARA, EPA and DHA needs to be predominantly supplied from dietary sources if high levels are to be incorporated into tissues.

Stahly (1984) reported that the supply of the essential fatty acids to pigs would normally be exceeded by diets containing maize or oats, but may not be adequate in young pig diets based on barley, wheat or milk protein sources. It has also been assumed that older mature animals have sufficient stores of essential fatty acids in muscle, liver and adipose tissue to resist deficiency for long periods (Cunnane, 1984). The recommendations for the supply of essential fatty acids from pig diets have changed little since these publications in the 1980's. Recently, Close and Cole (2000b) reviewed the literature and concluded that breeder diets should contain 0.7 g LIN/100 g feed and 0.5

g ARA/100 g feed. These authors did not mention any dietary requirement for omega-3 fatty acids by pigs.

In a comprehensive review of the literature on polyunsaturated fatty acids for the neonatal pig, Leskanich and Noble (1999) concluded that ARA, EPA and DHA have come to be regarded as essential for foetal and neonatal development in humans, and yet there remains little or no mention of required levels for these essential fatty acids in nutrient guidelines for pigs. Based on data available, these authors proposed a daily requirement for EPA + DHA of 2.9 g/day. Due to the lack of data available in pigs, these recommendations have been extrapolated from human dietary requirement recommendations.

1.3.2 Dietary sources of long-chain polyunsaturated fatty acids for pigs

Fish, particularly freshwater species, are a rich source of long-chain omega-3 fatty acids. In freshwater fish, this is likely due to the nature of the membrane fluidity at low temperatures (Corraze, 2001). The aquatic environment supplies an abundant source of PUFA that are bio-converted through fatty acid synthetase complex in a similar metabolic pathway as described above in mammals. In addition to endogenous sources through bioconversion of C18 fatty acids to longer chain PUFA, fish satisfy their nutrient requirements for essential fatty acids through their diet of other fish, shrimp and unicellular algae plant matter (Corraze, 2001). Fish, as in mammals, lack the necessary enzyme to synthesise LIN and ALA fatty acids, and although fish can convert C18 essential fatty acids to longer chain PUFA, the rate of bioconversion varies between species, freshwater fish being higher than marine fish (Corraze, 2001). As a combination of natural aquatic food sources and supplemented feed pellets, fish products, such as fishmeal and fish oil contain high levels of omega-3 and omega-6 PUFA (Table 1.1), with farmed freshwater fish having a high level of EPA and DHA (Opstveldt, 1984).

Table 1.1 Selected fatty acid content (g/100 g total fatty acid) of common dietary ingredients available as fats and oils for pigs[1]

	Linoleic acid C18:2	α-Linolenic 18:3 n-3	Long-chain PUFA's (>C20)	Total n-6	Total n-3
Tallow (beef)	3.1	0.6	0.3	3.1	0.6
Tallow (mixed)[1]	5.9	0.6	1.2	7.3	0.8
Canola oil	20.3	9.3	3.6	20.3	9.3
Soybean oil	51.0	6.8	0.2	51.0	6.8
Corn oil	59.0	0.7	<0.1	58.0	0.7
Safflower oil	74.1	0.4	<0.1	74.1	0.4
Olive oil	7.9	0.6	0.3	7.9	0.6
Anchovy oil	1.2	0.8	30.3	1.3	31.2
Herring oil	1.1	0.8	45.6	1.4	17.8
Menhaden oil	2.1	1.5	29.5	1.5	25.1
Commercial fish oil[1]	9.7	1.1	21.3	11.6	17.3

[1]Values sourced from Pearl (1995) of the Fats and Protein Research Foundation and the USDA Food Composition Standard Release 11 (1997) as cited in NRC (1998). See also (Rossi et al., 2010).
[2]Values analysed on commercial ingredient supplied Salmate™ (Feedworks Pty Ltd, Sunbury, VIC) by Nutrition and Functional Foods Laboratory, The University of Adelaide, Waite Campus, South Australia

Salmon oil supplemented diets have been shown to supply a lower proportion of total omega-3 fatty acids and proportionately more EPA than when equivalent levels of tuna oil are supplemented (Rooke et al., 2001c). Salmon oil supplementation also decreased arachidonic acid levels more than was observed with tuna oil in sow colostrum and piglet liver (Rooke et al., 2001c). Thus the dietary source of omega-3 long-chain PUFA can exert different physiological responses depending on the relative amounts of ARA, EPA and DHA.

1.4 The response to long-chain omega-3 fatty acids supplied from fish oil on reproduction and fertility in gilts and sows

The reproductive response to dietary omega-3 and omega-6 PUFA has been widely reported in cattle, however it has not been widely investigated in pigs. The pig has been frequently used as an experimental model for human growth and development (Leskanich and Noble, 1999) but little is published on the effects of altering dietary levels of omega-3 or omega-6 fatty acids on commercial reproduction outcomes and the mechanisms whereby a response, positive or negative, have occurred. Most of the published studies report on the effect of omega-3 supplementation to diets on foetal and neonatal development and immunological responses (Leskanich and Noble, 1999), however there is no recommendation for required levels of long-chain PUFA for pigs for reproductive processes.

1.4.1 Fertility responses to omega-3 polyunsaturated fatty acids in gilts and sows

A positive effect of increasing the supply of long-chain omega-3 fatty acids was first reported by Palmer et al. (1970) as an indirect consequence of using fish meal as an alternative dietary source of vitamins, minerals and amino acids. Whole menhaden fish meal was included at 6% of the diet formulated with corn-soybean meal, such that treatments were similar in protein. Energy intake was formulated to be slightly higher in the fish meal diets (increase of 2.3% in first gestation and 1.5% in second gestation). Dietary treatments were introduced 30 d prior to gilts being mated and continued over two reproductive cycles until the end of the second lactation. The inclusion of fish meal significantly increased the number of piglets born live and total born by an average of 0.9 and 0.8 pigs respectively, with the effect being greater in the second parity. The effect of the supplementation carried through to the number of piglets weaned. There was no effect on weaning weight or pre-weaning growth rate. The authors also reported a significant increase in the average daily gain of gilts and sows during gestation when fish meal was included in the diet, indicating that

actual dietary energy intake was higher in the gilts and sows on the fish meal diets. Although the difference in energy was small, it was not conclusive if the reproductive response was due to increased fatty acids supplied within the fishmeal or an energy response. There was also speculation that the improved reproduction reported by Palmer et al. (1970) may have been due to additional selenium in the fish meal which was absent in the basal diet fed to control sows, as later studies were unable to demonstrate an effect with 3% fish meal added to diets (Baker et al., 1974).

In 1995, Perez-Rigau et al. (1995) reported that there had been little research given to the possible importance of specific fatty acids on the developing embryo or foetus in pigs. The authors investigated the response to different fatty acid sources on embryo survival in pregnant gilts. Dietary treatment consisted of four diets based on corn-soybean and starch. The fatty acids evaluated were either coconut oil (high in saturated fatty acids); soybean oil (PUFA diet high in LIN and ALA); menhaden oil (PUFA diet high EPA and DHA); or the basal diet containing corn starch (monounsaturated fatty acids and LIN). Oil was added at 4% of the diet which did not contain any tallow. All diets were formulated to contain equal amounts of energy and protein, and vitamins and minerals. The fatty acid profile of the four diets showed that the dietary formulations created two diets similar in omega-6:omega-3 ratio at 25.5 and 27.3 for the starch base and coconut oil treatments, respectively, and a decreased ratio of 16.5 for the soybean oil treatment and 1.58 for the menhaden oil treatment. Perez-Rigau et al. (1995) reported a trend towards an increase ($P < 0.06$) in foetal survival in one of two trials using gilts fed menhaden oil (93.7%, n=8) compared to the control starch diet (78.8%, n=7). When the data was pooled for both trials, foetal survival was numerically highest (84.8%, n=20) in the menhaden oil treatment, whereas foetal survival was similar between the control and soybean treatment (77.1%, n=20 and 76.7%, n=22, respectively). Ovulation rate was unaffected by dietary treatment, as was age at breeding (natural oestrus), live weight at breeding and live weight at 42 d of pregnancy when gilts were slaughtered and

reproductive parameters were assessed. Dietary fatty acid profile was reflected in the conceptus tissue.

In addition to the trials on gilts fed the treatment diets three to four weeks before breeding and during gestation, Perez-Rigau et al. (1995) further evaluated the foetal survival response when parity 2 sows at weaning were fed either the starch control diet or the menhaden oil diet. Weaned sows previously fed an unsupplemented diet were allocated to treatment based on litter size and lactation weight loss and treatment diets were fed from day 1 postweaning. In sows supplemented postweaning, neither ovulation rate nor foetal survival were affected by dietary treatment (Perez-Rigau et al., 1995).

In other experiments when fish oil was only fed during pregnancy, there was no response to the litter size born, although there were responses to gestation length and foetal development (Rooke et al., 2001c; Mateo et al., 2009). From d 3 postmating, Rooke et al. (2001c) fed multiparous sows a wheat-based diet that contained no fish oil or 1.65% of fish oil. The dietary treatments were continued into lactation for a period of 25 d when all weaned sows were returned to a standard un-supplemented diet before being mated and fed control diets during the subsequent gestation. There was no significant effect of fish oil on litter size born following gestation feeding (12.3 and 11.9 total born in salmon oil and controls, respectively). Given that the omega-3 supplemented diets were introduced after ovulation/mating, this would be expected from other results. However, when sows continued on their dietary treatment through lactation until weaning, there was no difference between treatments in the subsequent parity on conception rates at three weeks postmating (mean of 95%), farrowing rates (mean of 78%) or litter size (12.4 and 11.8 total born; Rooke et al., 2001c). Over the period between weaning and mating, all sows were fed an un-supplemented diet and this may have limited the response to lactation omega-3 supplementation, as proposed by Mateo et al. (2009).

1.4.2 Commercial studies evaluating diet supplementation with omega-3 PUFA from fish oil

Two abstracts have been published where a commercial product containing equal amounts of EPA and DHA from marine sources (Fertilium™) was added as a supplement to corn-soybean diets offered prior to mating in gilts and sows (Spencer et al., 2004; Webel et al., 2004). In the study by Spencer et al. (2004), large numbers of gilts (317) were allocated 30 d prior to breeding to either a control group or one supplemented with 1.5% of the omega-3 product. They reported a significant increase from 10.4 live born in the un-supplemented treatment to 11.4 live born in those offered the additional omega-3 fatty acids (Spencer et al., 2004). No mention was made of conception rate or farrowing rate. Webel et al. (2004) fed primiparous and multiparous sows the same omega-3 PUFA supplement either 8 d before farrowing, during lactation and postweaning until mating after a 16 d lactation period, or fed for a shorter period of time just prior to farrowing to mating. They reported a significant increase in the subsequent litter born as piglets born live (10.9) and total born (12.0) in the omega-3 sows fed for 8 d prior to farrowing shed entry compared to the controls (10.2 and 11.3 born live and total born, respectively). The subsequent litter size born in the group fed the omega-3 supplement for the shorter duration of feeding prior to mating was similar to the un-supplemented sows, suggesting that there is an effect of feeding duration prior to ovulation/mating on the response to omega-3 supplementation. Weaning to oestrus interval and subsequent farrowing rate were not affected by dietary treatment. In a second experiment described in the same abstract, (Webel et al., 2004) reported there was no effect of omega-3 supplementation on ovulation rate, but there were significantly more live embryos (14.5 vs 11.9) in the sows fed the omega-3 supplement compared to un-supplemented sows when fed for 35 d or more prior to mating.

To determine the mechanism by which litter size was increased through omega-3 PUFA, Estienne et al. (2006) fed 1% Fertilium™ to pubertal gilts prior to mating and assessed ovulation rate and embryo number and survival at 27 d of gestation. In their experiment, gilts were synchronised using altronogest and mated artificially at oestrus following withdrawl of the progestin. There was no

difference between Controls or Omega-3 gilts in terms of ovulation (17.7 corpora lutea), embryo number (14.4) or embryo survival (79%) and they concluded that either they did not feed enough of the PUFA product or that the level of performance in the herd was already high and less responsive to dietary changes than when used previously by Webel et al. (2004).

1.5 Functional role of omega-3 polyunsaturated fatty acids in female reproduction

As interest increases in supplementing animal diets to alter the fatty acid composition of milk and meat as functional foods for human nutrition, effects on reproduction in livestock are becoming apparent. The improvement in reproduction reported in various studies suggests that specific fatty acids may be important for some physiological processes (Rossi et al., 2010). The mechanism by which omega-3 PUFA affect reproductive metabolism in mammals is largely unknown. In boars, there are some reported studies where fertility was improved by the addition of fish oil in diets fed to boars, due to enhanced spermatozoa quality (Rooke et al., 2001a). In other studies in pigs, feeding diets during gestation and lactation containing long-chain omega-3 PUFA from fish oil has improved piglet performance and preweaning growth and survival (for reviews see Leskanich and Noble, 1999; Rossi et al., 2010). However, there is a paucity of publications on the response of omega-3 PUFA on gilt and sow reproduction.

1.5.1 Effects of omega-3 polyunsaturated fatty acids on prostaglandin synthesis

Prostaglandin release during the end of the luteal phase of oestrous is necessary for the follicular phase to be initiated and ovulation to occur. Oxytocin and progesterone are secreted from the corpus luteum during the luteal stage of the oestrous cycle. Toward the end of the luteal phase, an increased pulse of oxytocin from the ovary acts as an endocrine signal to the uterus where $PGF_{2\alpha}$ is produced in the presence of progesterone. The prostaglandin $PGF_{2\alpha}$ causes regression of the corpus luteum on the ovary, or luteolysis. Concomitantly, progesterone secretion from the corpus

luteum rapidly declines over 1 to 2 days in preparation for ovulation. During pregnancy, the production of interferons from the blastocyst prevents the release of uterine $PGF_{2\alpha}$ by inhibiting the activation of oxytocin receptors on the uterine epithelium. In the sow, the main signal from the pig conceptus is oestradiol, an oestrogen that causes a re-direction of $PGF_{2\alpha}$ into the lumen of the uterus (Geisert et al., 2005; Ziecik et al., 2006). Increased oestradiol from the blastocyst is necessary for a reduction in uterine epithelial $PGF_{2\alpha}$ so that successful implantation and a continued secretion of progesterone can occur (Senger, 1997).

Chartrand et al. (2003) studied the effect of different fats on intrauterine prostaglandin synthesis during early pregnancy in gilts. They reported a reduction in $PGF_{2\alpha}$ and PGE_2 in plasma and the uterine fluid when linseed oil (high ALA) was supplemented compared to tallow oil. Long-chain omega-3 EPA and DHA from fish oil were not supplemented, although the authors reported a significantly higher level of EPA in plasma and endometrial tissues presumably due to biosynthesis from ALA. They concluded that the reduction in prostaglandin synthesis was due to the reduction in ARA and/or reducing the ARA substrate in the eicosanoid metabolism. A reduction in the synthesis of prostaglandins $PGF_{2\alpha}$ and PGE_2 has also been reported in rats fed diets containing high levels of EPA and DHA (Trujillo and Broughton, 1995).

In dairy cows, dietary supplementation with fishmeal or protected fish oil in dairy cow rations has been reported to have improved reproductive performance possibly through a reduction in the uterine secretion of the luteolytic prostaglandin, $PGF_{2\alpha}$. Mattos et al. (2002) fed cycling dairy cows menhaden fishmeal and reported a decrease in plasma $PGF_{2\alpha}$ following exogenous synchronisation with estradiol and oxytocin and supported results shown previously by Thatcher et al. (1997). From in-vitro cell culture studies using bovine endometrial cells, a dose-dependent inhibition of $PGF_{2\alpha}$ from EPA and DHA has been demonstrated (Mattos et al., 2001). In a further study comparing supplemented fish oil to olive oil (high LIN), Mattos et al. (2004) again reported a significant

reduction in plasma $PGF_{2\alpha}$ level when EPA and DHA was increased pre-partum and post-partum. However not all studies with fish oil derived EPA and DHA in cattle have reported a decrease in prostaglandins. Petit et al. (2002) compared calcium salts of linoleic acid to formaldehyde treated linseed oil (a balanced source of the LIN and ALA) and a combination of formaldehyde-linseed oil and fish oil which supplied EPA and DHA They reported higher levels of $PGF_{2\alpha}$ in cows on the fish oil supplemented diet after 22 d of feeding and suggested the discrepancy may have been due to relative differences in the omega-6:omega-3 ratio between studies (Petit et al., 2002).

Shortened gestation length and pre-term delivery in humans can result from high circulating levels of prostaglandins. Olsen et al. (1992) reported a longer pregnancy duration in women who took supplements of fish oil compared to olive oil or un-supplemented women, and that the response was higher in those women on fish oil supplements that had a low basal intake of omega-3 fatty acids. This has since been supported by prospective studies reviewed by Allen and Harris (2001) where pre-term deliveries were associated with high levels of omega-6 fatty acids and low omega-3 fatty acids. There is also evidence that increasing omega-3 PUFA from fish oil in the diet fed to sows through late pregnancy increases gestation length (Rooke et al., 2001c).

1.5.2 Effects of omega-3 and omega-6 polyunsaturated fatty acids on progesterone synthesis

Progesterone and oestrogen are fundamental hormones that determine the maternal recognition of pregnancy in all mammalian species. In order to prevent the loss of embryos and maintain pregnancy after maternal recognition signals, progesterone is produced from the ovarian luteal cells postmating (Geisert et al., 2005). Staples and Thatcher (1999) proposed that observed increases in fertility when supplemental fat sources were fed could be due to increased progesterone levels in early pregnancy. Early embryo loss is reported to be as high as 30 to 40% in commercial sows by 25 d of gestation (Dyck and Strain, 1983; Vonnahme et al., 1994). Low levels of circulating progesterone may be a cause of embryo mortality in the pig (Pharazyn et al., 1991) and a failure of

pregnancy may result if a reduction in the plasma progesterone is insufficient to support the conceptus in its uterine environment (Kirkwood and Thacker, 1988).

The synthesis of progesterone from ovarian luteal cells requires cholesterol as a dietary substrate and is under the influence of prostaglandins (Smith et al., 1994). Follicular granulosa and thecal cells change in the presence of the LH surge just prior to ovulation (Murphy et al., 2001). After ovulation, the membrane between granulosa and thecal cells on the inside of the follicle and surrounding the oocyte breaks down and the corpus luteum forms during luteinisation. Granulosa cells become the major luteal cell responsible for progesterone biosynthesis (LaVoie et al., 1997). Whilst dietary fats supply cholesterol, the precursor to progesterone, the influence of specific fatty acids on progesterone is likely through its regulation of synthesis (Smith et al., 1994) or regulation of progesterone cellular expression (Li et al., 1993).

Although $PGF_{2\alpha}$ causes the regression of the corpus luteum to allow a new wave of follicles to ovulate, not all prostaglandins have a luteolytic effect and reduce progesterone. Prostaglandin (PG) E1 and PGE_2 stimulate secretion of progesterone and are found in high levels in the uterine endometrium of pregnant pigs (Geisert et al., 1982; Kennedy et al., 1986). Wiesak et al. (1992) demonstrated in-vitro that by subjecting porcine luteal tissue to increasing levels of the prostaglandins $PGF_{2\alpha}$ and PGE_2, progesterone production increased, however when in the presence of LH, the effect of the prostaglandins was inhibitory. In-vitro studies have shown that PGE_2 can overcome the luteolytic action of $PGF_{2\alpha}$. In porcine granulosa cells, progesterone synthesis was increased when PGE_2 was combined with $PGF_{2\alpha}$ relative to in-vitro cell cultures with $PGF_{2\alpha}$ alone (Li et al., 1993). This was related to an increased mRNA gene expression for the two enzymes involved in cholesterol conversion to progesterone, $P450_{scc}$ and 3β-HSD. The role of prostaglandins during luteal development and maternal recognition of the conceptus is still to be fully understood, however there is evidence that PGE_2 can maintain progesterone synthesis by protecting granulosa cells from

the inhibitory effects of $PGF_{2\alpha}$. Relating this to a reproductive outcome, Kraeling et al. (1985) proposed that an inhibition of PGE_2 could disrupt the signal allowing the maintenance of pregnancy if embryo loss is too high. The possibility of a mechanism by which dietary omega-3 PUFA affects reproduction due to a positive effect on progesterone in pigs, either through regulation of progesterone production or on progesterone receptivity during implantation is not known.

In dairy cows, Burke et al. (1997) reported a greater proportion of cows fed diets supplemented with fishmeal had elevated progesterone concentration 48 h after oestrus induction with $PGF_{2\alpha}$. They concluded that the fishmeal prevented a complete regression of the corpora lutea. This model supports a hypothesis that fish meal and omega-3 fatty acids enhances the luteal function of the ovary, in turn improving conception and pregnancy rates observed by others in dairy cows (Bruckental et al., 1989; Armstrong et al., 1990; Carroll et al., 1994). However, Mattos et al. (2002) did not show any difference in progesterone level with menhaden meal supplementation. Trujillo and Broughton (1995) also reported that rats fed high levels compared to low levels of omega-3 fatty acids from fish oil had lower plasma progesterone levels after induced ovulation induction via hCG compared to unsupplemented controls. There are no published studies on the effect of diet changes to omega-3 PUFA on the progesterone response in pigs.

1.5.3 Effects of omega-3 polyunsaturated fatty acids on follicular growth and oocyte maturation

Follicle growth and development is important for reproductive performance, determining ovulation rate, oocyte quality and luteal support of the embryo after formation of corpora lutea. The follicle surrounding the oocyte provides a structure that controls the endocrine response during the luteal and follicular phases of the oestrous cycle, providing essential nutrients and metabolic support to the developing oocyte as it grows, and supports the signals that induces and maintain the intracellular maturation program of the oocyte (Moor and Dai, 2001; Telfer, 2001). Long-chain PUFA are normally present in the reproductive tissues and fluids of mammals, with the composition differing

between species (Khandoker et al., 1997). The pig oocyte has high levels of long-chain PUFA, particularly the omega-6 fatty acids, LIN and ARA, indicating a local role in actively producing prostaglandins (Homa et al., 1986; McEvoy et al., 2000). As well as the oocyte, Khandoker et al. (1997) reported 5-10% of total fatty acids are present as ARA in the fluid content of the follicle, uterine body and oviduct with the composition possibly being more important for fertilisation and embryo survival than shorter chain fatty acids.

There are numerous factors that affect follicle size and follicular development which have consequences on reproductive parameters, such as the time taken to resume oestrus postweaning (Lucy et al., 2001) and ovulation rate (Cox, 1997). Follicle growth has been shown to be positively related to energy balance (Cosgrove et al., 1992). Work in the pig has shown that follicles grow from 2 to 3 mm prior to weaning to 7 to 8 mm at the time of ovulation, and that fertile sows with a short weaning to ovulation interval have a high proportion of medium sized follicles postweaning (Lucy et al., 2001). Nutrition during lactation has an effect on both ovulation rate and embryo survival. A low plane of nutrition in mid to late lactation reduces embryo survival (Kirkwood et al., 1987; Cox, 1997), whilst an increase in feeding level in late lactation up until weaning improves oocyte maturation (Zak et al., 1997b). Ashworth and Antipatis (1999) proposed that the quality of the oocyte may be as important in determining embryo survival as the effect of nutrition in lactation on endocrine responses, such as LH, and poor oocyte quality has been implicated as a cause of unusually small, or runt embryos in early pregnancy (Quesnel et al., 2010).

There are no published studies which report on the direct effect of specific fatty acids or source of PUFA on folliculogenesis or oocyte quality in gilts or sows fed equal intakes of energy. In dairy cows, Petit et al (2002) reported that cows fed diets supplemented with a combination of linseed oil (high ALA) and fish oil (high EPA and DHA) tended to have more medium sized follicles whilst the corpora lutea were significantly larger in diameter compared to cows fed either a concentrate high in

LIN or high in saturated fat. In ewes, Zeron et al. (2002) supplemented diets with polyunsaturated fatty acids using a calcium salt of fish oil. More follicles and oocytes were recovered from ewes supplemented with fish oil-calcium salts than the un-supplemented controls and oocyte quality was improved when assessed for membrane integrity, cytoplasmic homogeneity and the level of cumulus layers (Zeron et al., 2002).

1.5.4 Effects of omega-3 polyunsaturated fatty acids on ovulation rate, embryo survival and conception rate

There are few published studies in animals fed the same level of energy where the response on ovulation rate and embryo survival has been due to specific changes to the fatty acid profile through dietary supplementation. In most cases where the effects on the ovary and embryo survival were measured, the use of synchronisation protocols using prostaglandin analogues and exogenous progesterone (altronogest) somewhat limit the interpretation of dietary fatty acid supplementation on ovulation rate on commercial fertility rates (Sklan et al., 1994; Estienne et al., 2006). The response to dietary supplements of omega-3 fatty acids on embryo survival and conception rate in pigs has been reported briefly by Webel et al. (2004). The number of live embryos recovered at an average of 30 d gestation was increased by 22% when mixed parity sows were fed a corn-soybean base diet plus a top-dress of a protected PUFA source for 35 d or more prior to mating during the prefarrowing, lactation and postweaning period. Webel et al (2004) found no effect of supplementation on ovulation rate. Estienne et al. (2006) also found no change in the ovulation rate of gilts supplemented with the same commercial omega-3 supplement used by Webel et al. (2004), however in the study in gilts by Estienne et al. (2006), there was no effect on embryo survival. In one of five trials, Perez-Rigau et al. (1995) observed a trend for higher embryo survival when 4% menhaden oil was used as an omega-3 supplement prior to mating in gilts compared with other PUFA sources including coconut oil, corn oil and soybean oil. When the same diets were fed to weaned sows before mating and during gestation, they reported no dietary response on embryo

survival over three separate trials (Perez-Rigau et al., 1995). There was no effect of dietary fat source on ovulation rate in either gilts or sows.

In experiments where there has been a positive response on embryo survival or litter size born when fish meal or fish oil diets are fed, conception and farrowing rates have also increased in some studies (Palmer et al., 1970; Perez-Rigau et al., 1995). Yet in the commercial study of Webel et al. (2004) where a large number of sows were allocated to treatment (n=209 and n=223 for PFA and Control respectively), there was no significant improvement in conception or farrowing rate. Evidence of a positive effect of long-chain omega-3 PUFA on conception rates has been more widely reported in dairy cows (Staples et al., 1998). It is often difficult to make conclusions on conception rates or farrowing rates from data sets where the numbers of animals were small, however the data sets of Palmer et al. (1970) and Perez-Rigau et al. (1995) provide some evidence for a positive effect of omega-3 fatty acids from fish oil.

1.5.5 Uptake of omega polyunsaturated fatty acids in the reproductive tract and conceptus

Several authors have reported that dietary supplementation of the long-chain omega-3 fatty acids, EPA and DHA in gilts and sows results in a higher concentration within follicular fluid, endometrial tissue and the conceptus implying that they have a specific function. Commencing at 150 d of age and continuing dietary treatments through pregnancy, Brazle et al. (2009) used a commercial long-chain omega-3 PUFA product to increase EPA and DHA levels. In their first experiment, fatty acid differences were recorded in the endometrium and chorioallantois sac at 40 d gestation when the omega-3 PUFA diet from fish oil was compared to a corn-soybean control with tallow and a diet supplemented with ALA from flax seed. In the endometrium, foetus and chorioallantois, DHA and DPA were increased in the diets containing fish oil and flax seed, but EPA was unaffected compared to the gilts fed a corn-soybean control. Brazle et al. (2009) suggested that there may be differences between tissues in the rate of biosynthesis from ALA to EPA and DHA, as the accumulation of DHA

seems to be important for foetal development at term (Rooke et al., 2001d). In a second experiment reported in their paper, Brazle et al. (2009) compared the uptake of long-chain fatty acids between gilts fed the diet containing the fish oil supplement and the control corn-soybean diet on d 11, 15 and 19 of gestation. Again there were dietary effects and day x diet interactions on fatty acid profile in the endometrial tissue, extra-embryonic membranes, and embryo tissue. Gilts fed the fish oil supplement recorded higher levels of EPA and DHA in the endometrium and extra-embryonic membranes, whilst DHA was increased in the foetal tissue. Between d 11 to 19, which covers the period of embryonic implantation (Waclawik et al., 2009), the concentration of DHA in the endometrium declined, whereas EPA increased between d 11 to 19. Although long-chain PUFA were affected by diet, there were no dietary effects on foetal number, diameter of the embryonic disc or foetal development. The authors concluded there may be critical requirements in early pregnancy where different fatty acids may affect conceptus development and survival, and these are different when compared to more advanced foetuses (Brazle et al., 2009).

McNeil et al. (2005) also assessed the fatty acid profile of average and small foetuses in pigs at 45, 65 and 100 d of gestation, but contrary to Brazle et al. (2009), they reported the concentrations of LIN and DHA in the phospholipid fraction were unchanged as pregnancy advanced. Foetal ARA levels were found to be higher at 65 d, but the magnitude of the increase was quantitatively small. Their study did not report the level of polyunsaturated fatty acids supplied in the diet so it is unclear what dietary level of EPA and DHA were fed.

1.6 Conclusion

Fatty acids are required for the normal functioning of mammals and are a component of phospholipids in cell membranes; a source of energy; and as a precursor for PUFA with a carbon chain length of C18 or longer. Because mammals lack the necessary desaturase complex required for the formation of double bonds beyond the C_9 position, they have an essential requirement for

fatty acids of C18 in the diet. The requirement for LIN and ARA as essential fatty acids is recommended to prevent deficiency symptoms. However there is growing evidence to suggest that mammals also optimise their health, development and reproduction when long-chain omega-3 polyunsaturated fatty acids are supplemented in the diet by the addition of fish products. Although there is synthesis from C18 precursors of the omega-6 and omega-3 fatty acids, the rates are very low and may be insufficient to adequately supply long-chain omega-3 fatty acids, such as EPA and DHA. Eicosanoid metabolism utilises the long-chain omega-6 fatty acid ARA as a precursor, however it is now known that the enzyme complexes involved in the synthesis of prostaglandins can utilise omega-3 EPA, which alters the physiological properties to those derived from ARA.

There is supporting evidence to suggest that long-chain omega-3 and omega-6 fatty acids can improve reproductive performance in livestock when increased in the diet. However there have been few experiments published on the effect of supplementing the diet with long-chain omega-3 PUFA on reproductive outcomes in pigs. Published and unpublished reports indicate that sows fed supplementary long-chain PUFA from fish oil or fish meal sources have a higher litter size and, in some studies, an improved conception rate and farrowing rate when compared to un-supplemented diets of corn-soybean. There are no reported studies where EPA and DHA is supplemented from fish oil added to wheat and cereal based diets commonly used in Australia.

Most of the experiments reported to date have been in cattle where calcium salts of PUFA or rumen bypass sources of protected linseed, flaxseed or fish oil have been supplementary fed prior to joining. It has been shown in numerous experiments that supplementing the diet with long-chain omega-3 from fish oil or fish meal increase conception rates and ovarian function, either follicle development or luteal function in dairy cows and sheep. To date, the functional role of long-chain omega-3 fatty acids on reproduction has not been comprehensively studied in the pubertal gilt or weaned sow. The response on embryo survival and ovulation rate and important commercial

reproductive outcomes such as the regulation of oestrous, litter size born and farrowing rate in gilts and sows fed diets containing fish oil has not been comprehensively investigated or reported. Establishing the mechanism by which long-chain omega-3 PUFA may regulate reproduction needs to be determined in pigs.

The fertility of the gilt and sow is a key driver of profitability within the pig industry and a nutritional strategy that enhances fertility through increased conception rate, litter size and lifetime productivity is therefore important. In particular, the mechanism(s) whereby long-chain omega-3 fatty acids from fish oil improve reproductive performance needs to be elucidated so that the consistency of response and the cost-effectiveness of supplementation strategies can be developed.

The aim of this thesis is to describe the series of experiments where long-chain omega-3 PUFA as fish oil were supplemented to isocaloric diets fed to gilts and multiparous sows and report on the reproductive responses. In the final chapters, experiments were designed to investigate possible mechanisms that may be involved.

Chapter 2 General Materials and Methods

2.1 Animals and treatment

All experiments were conducted at piggeries of Rivalea Australia Pty. Ltd. NSW, Australia, with the exception of the in-vitro experiment described in Chapter 9. The piggeries were located at Redlands Road, Corowa, NSW (Longitude, 146.336 E, Latitude 35.948 S). Animals were of Large White x Landrace composite breed, supplied from an internal replacement herd within Rivalea (PrimeGro™ Genetics, Corowa, NSW). Semen used was also supplied from PrimeGro™ Genetics, Corowa, NSW). Details of animal management during each experiment are described within each experimental chapter.

Experiment 9 was conducted at The University of Adelaide, Main Campus, Medical School, Frome Road, Adelaide. Tissue samples were collected from a commercial abattoir, Big River Pork, Murray Bridge, SA. No animals were subject to treatment prior to slaughter in the experiment described in Chapter 9.

2.1.1 Animal management

Live weight was recorded on individual animals using electronic weigh scales. Backfat P2 was measured by real-time ultrasound using a 3.5 mHz linear array probe (SC485 Piemedical, Esaote, Italy) 65 mm from the midline over the last rib as described by Close and Cole (2000a). Feed intake in lactation was calculated as the number of feeds given during lactation multiplied by the average weight delivered from a feed scoop of 3-kg capacity. Feed scoops were periodically weighed to confirm weights. Daily feed intake was calculated as the average offered from farrowing to weaning.

The age of sows is defined as parity. A parity 0 sow is defined as a sow prior to farrowing her first litter. Sows are described as to their parity at the commencement of the experiment. A gilt is

defined as a nulliparous sow and described as either a prepubertal (before observed first oestrus), pubertal (cycling female before mating), or pregnant gilt (first pregnancy). A multiparous sow is defined as being of parity 1 or greater. Irrespective of parity, subsequent reproductive performance is defined as the fertility outcome following the postweaning mating event. At d 30, sows were tested for pregnancy using real time ultrasound fitted with a 5 MHz transabdominal sector probe (Agri-Scan A-7, ECM, Angoulême, France). To confirm the diagnosis, sows that were tested to have had a failed pregnancy were re-tested the following week. A mating or pregnancy failure is defined as a failed pregnancy relating to the mating service that occurs during the experimental period. Farrowing rates are defined as the proportion or percentage of litters produced without gestation failure from the designated mating for a pubertal gilt or as the first mating attempt on the postweaning oestrus for a sow. All matings were performed by artificial insemination (AI) using an 80 mL dose containing 3 x 10^9 sperm cells supplied by PrimeGro™ Genetics, Rivalea Australia, NSW. The mating date was given as the day of first AI service. Further details as to the feeding management and husbandry of animals are described under the methodology section in each experimental chapter.

2.1.2 Treatment allocation

Treatments were allocated equally, either by live weight (gilts) within time block, or randomly within parity, average lifetime litter size born and within time block (weaned sows). Further details on the allocation of animals to treatments are described under the methodology section for each experimental chapter. All animals were individually identified with an ear tag which was recorded on an experimental data spreadsheet (Microsoft Office Excel™, 2007). Details such as mating date, farrowing date and details and fate of mating were recorded by stockmen on a herd recording database (PigFM, Rivalea Australia Pty. Ltd.).

2.1.3 Animal Ethics

All gilts and sows were used for experimental purposes following approval from Animal Ethics Committees based on National Health and Medical Research Council "Australian Code of Practice for the Care and Use of Animals for Scientific Purposes" (NHMRC, 2004). As all live animal studies were conducted at Rivalea Australia in NSW, approval was sought for each experiment under Rivalea Animal Care and Ethics Committee SPPL 111 under the following Animal Research Authority numbers and titles:

Chapter 3	01R103	An evaluation of potential ingredients to supply polyunsaturated fatty acids in the lactation diet and the effect on subsequent reproductive performance
Chapter 4	06R028C	Evaluation of omega-3 fatty acids supplementation as a nutritional approach to increased productivity and longevity in gilts and sows
Chapter 5	06R082C	The effect of omega-3 fatty acids from salmon oil on ovulation rate and embryo survival
Chapter 6	08R042C	Fertility responses to omega-3 fatty acids in lactation and postmating
Chapter 7	09R019C	Effect of omega-3 fatty acids during lactation and early gestation on ovulation and embryo survival in mature sows
Chapter 8	10R038C	Role of omega-3 fatty acids on embryo survival and progesterone

Experiments described in Chapters 3, 4, 5 and 6 were approved by The University of Adelaide Animal Ethics Committee under the authority number M-025-2006 and the amendment M-025-2006A "Functional role of omega fatty acids for reproduction in the breeding gilt and sow". Experiments described as in Chapters 7, 8 and 9 were approved by The University of Adelaide AEC

as project M-2009-147 “Fertility responses to omega-3 fatty acids from fish oil in lactation and early gestation in multiparous sows”.

2.2 Diets and ingredients

Diets used in experiments described in Chapters 3 – 8 were manufactured at Rivalea Australia Pty. Ltd., Honour Avenue, Corowa, NSW. Ingredients were combined after hammer-milling of grains and micro-ingredients were added during mixing. Supplemented diets were achieved by partial replacement of tallow with fish oil on a weight basis. Tallow and fish oil was added through a post-pellet spray application. All diets were prepared as a 4-mm pellet at 85^{o}C before cooling and delivery. Diets were formulated by nutritionists in consultation with the author. Diets were prepared as 3-t batches and delivered in bags (20 kg) or in bulk to designated silos on the farm.

Tallow was supplied from Biodiesel Producers Pty. Ltd., Barnawartha, VIC and was derived from porcine and ruminant sources. In all diets, the tallow was stabilized using a liquid antioxidant (ethoxyquin as Rendox™, 250 mg/kg of diet, Kemin Industries, Des Moines, IA) during storage. Fish oil was sourced as liquid Salmate™ from Feedworks Pty. Ltd., Sunbury, VIC for experiments in Chapter 3 – 6. The main source of oil was from salmon in North America and the oil was manufactured in the US by Ballard Industries, OH. The product contained natural antioxidants from plant sources. For experiments in Chapter 7 and 8, fish oil was supplied by Optigen Ingredients™, Port Adelaide. There was no selenium or vitamin E included as an additive to either products. Mercury levels were tested as part of USDA product manufacture requirements and all product was sold with mercury levels < 0.01 mg/kg. During diet manufacture, an antioxidant (ethoxyquin as Endox™, 100 mg/kg of diet, Kemin Industries, Des Moines, IA) and vitamin E as α-tocopherol (120 mg/kg of diet, equivalent of 179 I.U. per kg) was added in the vitamin and mineral premix supplied at

the same rate in all diets as a preservative. Further dietary information and feeding management is described under the methodology section of each experimental chapter.

2.3 Experimental feeding regimen

The feeding program of supplementation was developed as the thesis progressed. All experimental diet composition and fatty acid profile where measured is reported in detail within each experimental chapter. In all experiments, animals fed diets containing omega-3 PUFA from fish oil were compared to animals fed an unsupplemented diet. In Chapter 3, the first experiment of the thesis involved feeding an omega-3 supplemented lactation diet (3 g fish oil/kg of diet) to multiparous sows from entry to the farrowing shed prefarrowing and continued through lactation until weaning. Following weaning, dietary treatments ended and all sows were fed a commercial unsupplemented gestation diet. In Chapter 4, gilts were fed an omega-3 supplemented gilt development diet (3 g fish oil/kg of diet) before mating for either 6 weeks duration or 3 weeks duration. In the slaughter experiment in gilts described in Chapter 5, pre-pubertal gilts were fed diets containing either 3 or 10 g fish oil/kg of diet and these diets continued to be fed after mating. From Chapter 6 onwards, multiparous sows were used as the experimental animal. In Chapter 6, sows were fed a supplemented lactation diet with 3 g fish oil/kg of diet from prefarrowing through lactation and continued after weaning up to mating. During gestation, all sows were fed an unsupplemented gestation diet until slaughter. In Chapter 7 and 8, multiparous sows were again fed a supplemented lactation diet (3 g fish oil/kg of diet) prefarrowing and continued through lactation to mating. In a 2 x 2 factorial design, experimental sows were then divided into groups and fed either a supplemented gestation diet (6 g fish oil/kg of diet) or an unsupplemented gestation diet for four weeks postmating. In Chapter 7, sows were assessed at the subsequent farrowing, while in Chapter 8 sows were slaughtered at d 23.

2.4 Fatty acid analysis

Feed and plasma samples were analysed for fatty acids by the Nutrition and Functional Foods Laboratory, The University of Adelaide, Waite Campus, Urrbrae, SA.

2.4.1 Feed samples

Feed samples were collected as 200 g samples at the time of the commencement of the experiment and kept at -20^{o}C until being sent for analysis. Three samples (1 g) were taken of each diet and were pooled for analysis of fatty acids using gas chromatography. After grinding to a particle size of approximately 1 mm using a mortar and pestle, the sample was mixed with 1.25 mL cold 0.9% saline and 3 mL of methanol. The sample was homogenized and allowed to stand for 10 min at room temperature, then 6 mL of chloroform was added and the sample shaken and allowed to stand for a further 5 min. The samples were then centrifuged for 10 min at 1,560 g at room temperature, and the chloroform-bound organic solute was removed and the extracted lipid fraction evaporated through a vacuum concentrator and after evaporation, the total lipid weight was determined. Approximately 10 mg of lipid was transferred to a scintillation vial containing 5 mL 1% sulfuric acid in methanol (vol/vol) and allowed to methylate for 3 h at 70^{o}C. After cooling, the resulting fatty acid methyl esters were extracted by adding 750 µL of distilled water and 2 mL heptane, agitated and allowed to stand at room temperature until layers separated. Fatty acid methyl esters in heptane were then transferred to 2-mL chromatography vials containing 1 to 2 grains of anhydrous sodium sulfate. Fatty acids were measured by gas chromatography from 2 µL injection to a gas chromatograph (6890GC, Hewlett Packard, Palo Alto, CA) fitted with a 50-m capillary column (BPX-70, SGE Analytical Science, Melbourne, VIC). Helium was the carrier gas and the split-ratio was 20:1. The injection port temperature was 250^{o}C and the detector temperature was 300^{o}C. The initial column temperature was 140^{o}C and increased to 220^{o}C at a rate of 5^{o}C/min. The identity of

each fatty acid peak was determined by comparison of peak retention time to authentic lipid standards (463 fatty acid methyl ester, Nu-Chek, Elysian, MN). The relative amount of each fatty acid (% of total fatty acids) was quantified by integrating the area under the peak and dividing the result by the total area for all fatty acids extracted. The fatty acid profile of the diets was expressed in g fatty acid/100 g total fatty acids in the results section for each experimental chapter.

2.4.2 Plasma samples

Blood samples were collected from the jugular vein of restrained sows using a 10 mL vacuum tube with lithium heparin (BD Vacutainer™, Franklin Lakes, NJ) fitted with a 38 mm (1 ½ inch) 16 gauge needle. The technique is described by Rivalea ACEC Standard Operating Procedure 36 and is based on the technique described by Framstad (2000). After collection, samples were stored on ice packs and relocated to the laboratory at the Research and Innovation Unit, Rivalea Australia, Corowa, where they were centrifuged at 2000 g for 5 min. Plasma was poured off and collected into a separate 5 mL tube, labelled by sow number and stored at -20^{o}C until being sent for analysis.

After thawing, 1 mL of plasma samples were extracted in duplicate and mixed with 0.5 mL 0.9% saline and 2 mL of methanol. The sample was shaken and allowed to stand for 5 min at room temperature, then 4 mL of chloroform was added and the sample shaken and allowed to stand for a further 10 min. The samples were then centrifuged for 10 min at 1,560 g at room temperature, and the chloroform-bound organic solute was removed by pipette and transferred to a 20 mL glass scintillation vial. The extracted lipid fraction was passed through a nitrogen evaporator and the total lipid weight was determined. 5 mL 1% sulfuric acid in methanol (vol/vol) was added to the extracted lipid and allowed to methylate for 3 h at 70^{o}C agitating every 30 min. After cooling, the resulting fatty acid methyl esters were extracted by adding 250 µL of distilled water and 0.5 mL heptane, agitated and allowed to stand at room temperature until layers separated. Fatty acid methyl esters in heptane were then transferred by glass pipette to a 2-mL chromatography vial containing 1 to 2

grains of anhydrous sodium sulfate. Fatty acids were measured by gas chromatography from 2 µL injection to a gas chromatograph (6890GC, Hewlett Packard, Palo Alto, CA) fitted with a 50-m capillary column (BPX-70, SGE Analytical Science, Melbourne, Australia). Helium was the carrier gas and the split-ratio was 20:1. The injection port temperature was 250^{o}C and the detector temperature was 300^{o}C. The initial column temperature was 140^{o}C and increased to 220^{o}C at a rate of 5^{o}C/min. The identity of each fatty acid peak was determined by comparison of peak retention time to authentic lipid standards (463 fatty acid methyl ester, Nu-Chek, Elysian, MN). The relative amount of each fatty acid (% of total fatty acid mass) was quantified by using the 463 standard to convert peak areas to a concentration value and dividing the result by the total amount for all fatty acids extracted. The fatty acid profile of the diets was expressed in g fatty acid/100 g total fatty acids in the results section for each relevant experimental chapter.

2.5 Statistical analysis

Data was analyzed using a general linear model (GLM) ANOVA for treatments in a randomized design model assuming equal variance (Sokal and Rohlf, 1981). The analyses were performed using SPSS 18.0 (SPSS Inc., Chicago, IL.) using sow as the experimental unit. Independent variables of dietary treatment, parity and block were assessed as main effects and included as factors in the univariate GLM model when significant. The main effect of treatment was analyzed using Bonferroni adjustment to confidence intervals for multiple treatment comparisons. Pairwise comparisons were performed using Least Significant Difference t-test. Blood sample parameters for fatty acid content and progesterone as applicable were analysed as repeated measures. Mean values are expressed as mean ± standard error of the mean (SE). Farrowing rate, the proportion of sows resuming oestrus within 7 days after weaning, and sow removal categories were analyzed as chi-square. Subsequent litter size was analyzed with parity, lactation length and previous average

lifetime litter size used as independent co-variate factors. Week of mating was analyzed for main effect on subsequent litter size. Probability *P* values < 0.05 were described as significant and *P* < 0.10 were used to describe trends.

Chapter 3 Effect of omega-3 fatty acid supplementation on reproductive performance in sows

3.1 Introduction

The dietary supply of the fatty acids LIN (C18:2) and ALA (C18:3) is essential for mammals as they lack the necessary desaturase enzymes to synthesize longer chain PUFA from shorter chain fatty acids (Enser, 1984). These longer chain PUFA, which include ARA (C20:4, n-6) and EPA (C20:5, n-3), are substrates required to synthesize prostaglandins (Caughey et al., 2005). Supplementation of animal diets with fatty acids derived from fish products has been previously shown to improve reproductive performance in the dairy cow (Bruckental et al., 1989; Armstrong et al., 1990; Carroll et al., 1994; Burke et al., 1997), and the ewe (Zeron et al., 2002). Omega-3 PUFA from fish oil have been shown to improve the development of the brain and immunity of neonatal piglets (Leskanich and Noble, 1999), however there is little information regarding the effect of omega-3 PUFA on fertility in sows. Palmer et al. (1970) reported an increase in litter size born in first and second parity sows when diets containing fish meal were fed from first mating continuing through gestation and lactation until the second parity. Perez-Rigau et al. (1995) studied the effect of adding 4% menhaden fish oil on foetal survival in gilts and sows and were compared with diets formulated with vegetable oils and starch. Gilts were fed diets during puberty from 160 d of age to six weeks postmating and sows from d 1 postweaning until slaughter six weeks after mating. However their results were inconsistent. In weaned sows fed a supplemented diet of fish oil there was no effect of omega-3 PUFA on embryo survival, whilst in one of two gilts trials the authors reported a trend for increased embryo survival in gilts fed the fish oil diet compared to the starch control.

Nutrition during lactation up to weaning has been shown to affect oocyte quality in pigs (Zak et al., 1997a) and conceptus survival and growth (Ashworth and Antipatis, 1999). In isocaloric diets, EPA and DHA (C22:6 n-3) have been related to increases in follicle size in cattle (Petit et al., 2002) and elevated DHA levels in the conceptus and endometrium in early gestation in gilts (Brazle et al., 2009). Increasing omega-3 PUFA prefarrowing and during lactation could improve oocyte quality and embryo survival, and consequently pregnancy rate and litter size. Including fish oil in the lactation diet of sows as such may improve sow productivity. The aim of the present study therefore was to determine whether feeding sows a diet containing omega-3 PUFA from fish oil 8 d before farrowing and continuing through lactation to weaning at 19 d would increase subsequent farrowing rate and litter size.

3.2 Materials and Methods

3.2.1 Animals and diets

Three hundred and twenty eight Large White x Landrace F1 sows were allocated to the experiment at 107.7 ± 0.1 d of pregnancy (mean ± SE) and ranged from d 100 to 114. Sows ranged in parity before farrowing from 0 to parity 7, with an average of 1.95 ± 0.09 across treatment groups (P = 0.529). The control group (Control, n = 169) were fed a diet containing 13.9 MJ DE/kg; 171 g crude protein/kg; and 9 g total lysine/kg. The diets were based on the cereal grains wheat and barley, dehulled lupins, and the byproducts tallow, rice pollard and millrun (wheat middlings) and were typical of ingredients used in commercial formulations at the piggery (Table 3.1). Sows fed a diet supplemented with fish oil (Omega-3, n = 159) received a diet of similar composition and supplemented with 3.3 g fish oil/kg of diet as a partial replacement for tallow. The level was based on a recommended daily intake of 2.9 g of EPA + DHA (Leskanich and Noble, 1999) and was fed at the level recommended by the supplier. Additions of tallow and oil were within levels typically used

in commercial formulations (Close and Cole, 2000a). Diet manufacture and ingredient suppliers of tallow and fish oil are detailed in Chapter 2.

3.2.2 Feeding and animal management

Sows were moved from gestation accommodation in group pens into the farrowing unit in their 15th week of gestation. Sows were randomly allocated to treatment and fed 3 kg of their treatment diet once a day at 0700 hours prior to and on the day of farrowing. Within 14 h of farrowing, litter size as number born live and dead were recorded, and the total litter weighed (born live only). Cross-fostering of piglets was practiced within dietary treatment between 18 h and 2 d after farrowing. Litters were weighed 3 d after farrowing and again at weaning (18.7 ± 0.1 d). Any sow that had her entire litter replaced due to illthrift (*E. coli,* poor weight gain) or removed due to mortality or agalactia was recorded and excluded from the data analysis during lactation, postweaning and subsequent litter performance. There was no creep feed provided to the piglets during lactation and each creep area was heated with a 175 W heat lamp.

From the day after farrowing, sows were offered their treatment diet up to four times a day to appetite. Feed refusal by sows was noted but feed residues were not weighed. Feed delivery was adjusted to the sow's appetite on a daily basis during lactation commencing from d 1. After weaning at 18.7 ± 0.1 d of age, all sows were housed in gestation stalls and fed a commercial gestation diet containing no fish oil or fish meal once a day (12.9 MJ DE/kg; 130 g crude protein/kg; 5.6 g total lysine/kg) at 2.7 kg/d and were mated on their first observed postweaning oestrus. Sows were detected in oestrus by fence line contact of a mature boar once a day and were artificially inseminated in an am/am service, 24 h apart. After insemination, sows were housed in groups (3 m^2/sow) according to their week of mating. Experimental treatments were housed within the same group during gestation. Sows were fed 2.5 kg of the commercial gestation diet once a day from d 2 of gestation until entry to the farrowing unit for their subsequent litter. Mated sows were checked for

oestrus from 18 d of mating using a mature boar and applying back pressure to the sow. At d 30, sows were tested for pregnancy. Reason for sow removal, subsequent farrowing rate, and subsequent litter size born were recorded within 24 h of birth. Total litter size is defined as the sum of live born and still born foetuses.

3.2.3 Fatty acid analysis

Three samples (1 g) were taken of each treatment diet (Control and Omega-3) and were pooled for analysis of fatty acids using gas chromatography at the Nutrition and Functional Foods Laboratory, The University of Adelaide, Waite Campus, South Australia (Table 3.2). The method is described in Chapter 2. The fatty acid profile of the treatment diets in g/100 g of total fatty acids extracted is summarized in Table 3.2.

3.2.4 Statistical analysis

Data was analyzed using a general linear model (GLM) ANOVA for treatments in a randomized design model assuming equal variance (Sokal and Rohlf, 1981). The analysis was performed using SPSS v18.0 (SPSS Inc., Chicago, IL.) using sow as the experimental unit. Experimental diets were fed to sows prefarrowing and during lactation in individual facilities. Independent variables of dietary treatment, parity and block were assessed as main effects. Mean values are expressed as mean ± standard error of the mean (SE). Farrowing rate, the proportion of sows resuming oestrous within 7 d after weaning, and sow removal categories were analyzed as chi-square. Subsequent litter size was analyzed with parity, lactation length and previous average lifetime litter size used as independent co-variate factors. Week of mating was analysed for main effect on subsequent litter size. Probability values < 0.05 were described as significant, and $P < 0.10$ were used to describe trends.

3.3 Results

3.3.1 Dietary levels of omega-3 PUFA

Fatty acid analyses confirmed that when 3 g of fish oil per kg of diet replaced the equivalent quantity of tallow, the dietary level of EPA and DHA was increased five-fold compared to the Control lactation diet (Table 3.2). The chemical analysis of crude fat for samples of Control diet and Omega-3 diet was 5.52 ± 0.12% and 5.62 ± 0.07%, respectively. Analyzed crude fat was within 5% of the calculated fat levels. Based on the chemical analysis levels and sow feed intake (Table 3.4), the Omega-3 diet provided 2.84 g/d of EPA and DHA. The Control diet provided 0.21 g/d EPA and DHA.

3.3.2 Effect of diet on lactation performance

The duration of dietary treatment before farrowing was not different between treatment groups (8.2 ± 0.1 d). The number of piglets born live, still born and average piglet birth weight at the initial farrowing was unaffected by dietary treatment (Table 3.3). Litter size born increased ($P < 0.001$) and birth weight tended to increase ($P = 0.091$) with parity, but there was no diet x parity interaction for for either measure (data not shown).

By d 3 when piglet weight gain assessment commenced, the number of piglets on each sow was similar between treatments (9.7 ± 0.1). There was no effect of dietary treatment prefarrowing and lactation on piglet weight gain and weaning weight (Table 3.4). Piglet weaning weight was lower ($P < 0.001$) in first-litter sows compared to all other parities (5.5 ± 0.1 kg vs 6.1 ± 0.1 kg), as was piglet weight gain (0.232 ± 0.005 kg/d vs 0.262 ± 0.003 kg/d, respectively, $P < 0.001$, data not shown). There was no significant diet x parity interaction on piglet weaning weight ($P = 0.667$) or weight gain ($P = 0.298$). The litter size weaned was unaffected by dietary treatment (9.2 ± 0.1, $P = 0.769$). Feed intake of sows over the lactation period was unaffected by dietary treatment (Table 3.4). Intake was less in first-litter sows compared to other parities (6.9 ± 0.1 kg vs 8.2 ± 0.1 kg/d, $P < 0.001$, data not

shown). Because all sows commenced feeding their respective treatment diet on the same day prior to farrowing and finished on the same day at weaning, the duration of treatment feeding from the late pregnancy to weaning was equal at 27 d for both treatments. During lactation, 10 Control sows and 14 Omega-3 sows were removed from the study (χ^2 1.01, P = 0.316) for use as foster sows nursing runt litters or fostered piglets of a dissimilar treatment; sow agalactia; or mortality.

3.3.3 Reproductive performance at the subsequent parity

There were no differences between dietary treatments in the proportion of sows mated within 7 d following weaning (83.7% vs 90.2% for Control and Omega-3 sows, respectively: χ^2 2.54, P = 0.110). Weaning to oestrus interval was also similar between treatment groups of sows (Table 3.5). In total, 147 Control sows and 132 Omega-3 sows were mated on their first postweaning oestrus. After weaning, 12 sows on the Control treatment were culled from the experiment due to anoestrus (n= 4), sow mortality (n = 2) and structural soundness (n = 6). Thirteen sows from the Omega-3 treatment were culled postweaning due to anoestrus (n = 4) and structural soundness (n = 9). Sow removals postweaning between treatments were not different (χ^2 0.20, P = 0.653).

Pregnancy rate at 30 d and farrowing rate was unaffected by dietary treatment (Table 3.5). Forty one Control sows and 35 Omega-3 sows failed to maintain pregnancy and farrow (χ^2 0.07, P = 0.797). Reproductive failure described as return to oestrus (d 0 to 30), negative pregnancy test by ultrasound (d 30 to 42), abortion, or late term pregnancy loss (d 42 to 114) accounted for the majority of losses (38 Control sows vs 33 Omega-3 sows) and was similar between Control sows and Omega-3 sows (χ^2 0.03, P = 0.871).

The number of piglets born live from Omega-3 sows was higher (P = 0.013) in the subsequent parity (10.3 ± 0.3) than that for Control sows (9.3 ± 0.3; Table 3.5). The percentage of stillborn piglets as a proportion of subsequent total litter size was not different between treatments and averaged 3.9 ±

0.5%. Subsequent total litter size born was also higher in sows on the Omega-3 treatment. Overall, the litter size from Omega-3 sows was increased without a significant reduction in litter size variation (Figure 3.1). To confirm this, a comparison of the coefficient of variation values for the subsequent total born did not differ between treatments (0.269 vs 0.326, for Omega-3 and Control sows, respectively; $P = 0.272$).

Table 3.1 Ingredient and calculated nutritional composition of lactation diets fed prefarrowing and during lactation (as fed basis) to sows allocated to either a control diet (Control) or a diet containing omega-3 PUFA from 3 g fish oil/kg of diet (Omega-3)[1]

	Treatment diet		Commercial
Ingredient, g/kg	Control	Omega-3	Gestation diet
Wheat	448.0	505.0	450.0
Barley	163.0	202.0	182.0
Millrun (wheat middlings)	100.0	76.0	290.0
De-hulled lupin (*L. angustifolius)*	150.0	82.0	-
Rice pollard	50.0	-	-
Canola meal	-	20.0	19.3
Meat meal	20.0	50.0	10.0
Tallow[2]	18.7	22.0	15.0
Blood meal	11.7	10.0	-
Di-calcium phosphorus	15.1	5.0	10.0
Water	10.0	10.0	10.0
Limestone	4.00	5.0	7.3
Salt	3.5	3.5	3.0
Fish oil[3]	-	3.3	-
L-lysine HCl	2.3	2.4	1.8
Mineral vitamin premix[4]	2.3	2.3	1.3
DL-methionine	0.7	0.5	-
Threonine	0.7	0.9	-
Phytase	0.1	0.1	0.1
Calculated nutrient analyses, g/kg			
Digestible energy, (MJ/kg)	13.9	13.9	13.0
Crude protein	171.0	173.0	128.0
Crude fat[5]	57.0	54.0	42.0
Crude fiber	37.0	35.0	48.0
Calcium	8.6	8.6	7.9
Total phosphorus	8.0	6.3	7.0
Available phosphorus	5.3	5.3	5.0
Lysine	8.9	9.0	6.0
Ileal digestible lysine, (g/MJ DE)	0.57	0.57	0.40
Methionine	2.7	2.8	2.0
Methionine + cystine	5.9	6.0	4.8
Threonine	6.2	6.5	4.1
Valine	8.3	8.2	5.8
Isoleucine	6.2	6.0	4.3
Tryptophan	0.2	0.2	0.2

[1]Diets commenced 107.7 ± 0.1 d of gestation and fed 3 kg/d then continued *ad libitum* during lactation until weaning at 18.7 ± 0.1 d.

[2]Tallow supplied by Biodiesel Producers, Barnawartha, VIC.

[3]Fish oil supplied by Feedworks, Sunbury, VIC.

[4]Premix provided the following nutrients (per kg diet as fed): copper, 20 mg; iron, 80 mg; organic iron, 50 mg; manganese, 40 mg; zinc, 100 mg; iodine, 1 mg; selenium inorganic, 0.15 mg; organic selenium, 0.15 mg; chromium picolinate, 3.2 mg; betaine, 2.5 g; antioxidant (Endox), 100 mg; vitamin A (retinol), 15,000 I.U.; vitamin D (cholecalciferol), 1,500 I.U.; vitamin E (α-tocopherol), 120 mg; vitamin B_2 (riboflavin), 3.5 mg; vitamin B_6 (pyridoxine), 2 mg; vitamin B_{12} (cyanocobalamin), 0.02 mg; biotin, 0.2 mg; folic acid, 0.5 mg; niacin, 65 mg; pantothenic acid, 5 mg.

[5]Crude fat determined by chemical analysis was 55.2 ± 1.2 g/kg, Control diet and 56.2 ± 0.7 g/kg, Omega-3 diet

Table 3.2. Fatty acid composition (g/100 g total fatty acids as fed basis) of lactation diets[1] fed prefarrowing and during lactation (as fed basis) to sows allocated to either a control diet (Control) or a diet containing omega-3 PUFA from 3 g fish oil/kg of diet (Omega-3)[2]

Fatty acid (Common name)	Control	Omega-3
C16:0 (palmitic acid)	18.3	19.1
C16:1 (palmitoleic acid)	0.23	0.29
C18:0 (stearic acid)	8.05	9.60
C18:1 (oleic acid)	28.0	26.9
C18:2 n-6 (α-linoleic acid)	35.6	31.0
C18:3 n-6 (γ-linolenic acid)	0.01	0.02
C18:3 n-3 (α-linolenic acid)	2.14	2.27
C20:4 n-6 (arachidonic acid)	0.12	0.19
C20:5 n-3 (eicosapentaenoic acid)	0.02	0.24
C22:5 n-3 (docosapentaenoic acid)	0.05	0.19
C22:6 n-3 (docosahexaenoic acid)	0.03	0.40
Total n-6	35.9	31.5
Total n-3	2.38	3.32
n-6:n3 ratio	15.1	9.5
Total saturated fatty acids	29.0	31.9
Total transaturated fatty acids	0.16	0.21

[1]Ingredient and nutrient composition described in Table 1.
[2]Diets commenced 107.7 ± 0.1 d of gestation (3 kg/d) and continued *ad libitum* during lactation until weaning at 18.7 ± 0.1 d.

Table 3.3 Litter size and birth weight of piglets[1] born to sows fed prefarrowing and during lactation either a control diet (Control) or a diet containing omega-3 PUFA from 3 g fish oil/kg of diet (Omega-3)

	Treatment[2]			
Item[3]	Control	Omega-3	SEM	*P* value
Born live, pigs/litter	10.1	10.4	0.2	0.357
Still born, pigs/litter	0.6	0.5	0.1	0.198
Total born, no.	10.9	11.2	0.2	0.457
Pig birth wt, kg /litter	1.46	1.44	0.02	0.673

[1]Litters were weighed within 24 hours of birth.
[2]Sows fed diets containing no fish oil (Control, n = 169) or a diet containing 3 g fish oil/kg of diet (Omega-3, n = 159) commencing 107.7 ± 0.1 d of gestation (3 kg/d) and continued *ad libitum* during lactation until weaning at 18.7 ± 0.1 d.
[3]Estimated marginal mean values with parity (1.9 ± 0.1) and period of treatment feeding prior to farrowing (8.2 ± 0.1 d) included as covariate factors.

Table 3.4 Average piglet weight at day 3 and weaning, piglet daily gain and average daily intake of sows fed prefarrowing and during lactation either a control diet (Control) or a diet containing omega-3 PUFA from 3 g fish oil/kg of diet (Omega-3)

	Treatment[1]			
Item	Control[2]	Omega-3[3]	SEM	*P* value
Pig d 3 live wt, kg[4]	2.0	1.9	0.1	0.146
Pig ADG d3 - wean, kg/d[5]	0.256	0.256	0.003	0.868
Pig weaned wt, kg/piglet[5]	6.0	5.9	0.1	0.318
Sow ADFI, kg/d[5]	7.8	7.9	0.1	0.624

[1]Sows fed diets containing no fish oil (Control) or a diet containing 3 g/kg of fish oil (Omega-3) commencing 107.7 ± 0.1 d of gestation (3 kg/d) and continued *ad libitum* during lactation until weaning at 18.7 ± 0.1 d.
[2]Control litters, n = 159 sows.
[3]Omega-3 litters, n = 145 sows.
[4]Estimated marginal mean values with parity (1.9 ± 0.1) and period of treatment feeding prior to farrowing (8.2 ± 0.1 d) included as covariate factors. Litter size standardized between treatments (9.7 ± 0.1).
[5]Average daily feed intake as estimated marginal mean values with parity (1.9 ± 0.1), period of treatment feeding prior to farrowing (8.2 ± 0.1 d) and weaning age (18.7 ± 0.1 d) included as covariate factors.

Table 3.5 The subsequent reproductive performance of Control and Omega-3 sows following weaning and cessation of dietary treatments

Item	Treatment[1]		SEM	*P* value
	Control[2]	Omega-3[3]		
Wean to oestrus, d[4]	7.8	6.3	0.4	0.130
Number sows mated	147	132		
Conception to 30 d, no.	110	100	χ^2 0.03	0.858
Conception rate, %	74.8	75.8		
Sows farrowed, number sows	106	97	χ^2 0.07	0.797
Farrow rate, %	72.1	73.5		
Subsequent born live[5]	9.3	10.3	0.2	0.013
Subsequent total born[5]	9.7	10.7	0.2	0.022

[1]Sows fed diets containing no fish oil (Control) or a diet containing 3 g/kg of fish oil (Omega-3) commencing 107.7 ± 0.1 d of gestation (3 kg/d) and continued *ad libitum* during lactation until weaning at 18.7 ± 0.1 d.

[2]Control litters, n = 147 sows mated postweaning.

[3]Omega-3 litters, n = 132 sows mated postweaning.

[4]Estimated marginal mean values with parity (1.9 ± 0.1) and weaning age (18.7 ± 0.1 d) included as covariate factors.

[5]Estimated marginal mean values with parity (1.9 ± 0.1), previous lifetime average total born (10.9 ± 0.1) and weaning age (18.7 ± 0.1 d) included as covariate factors.

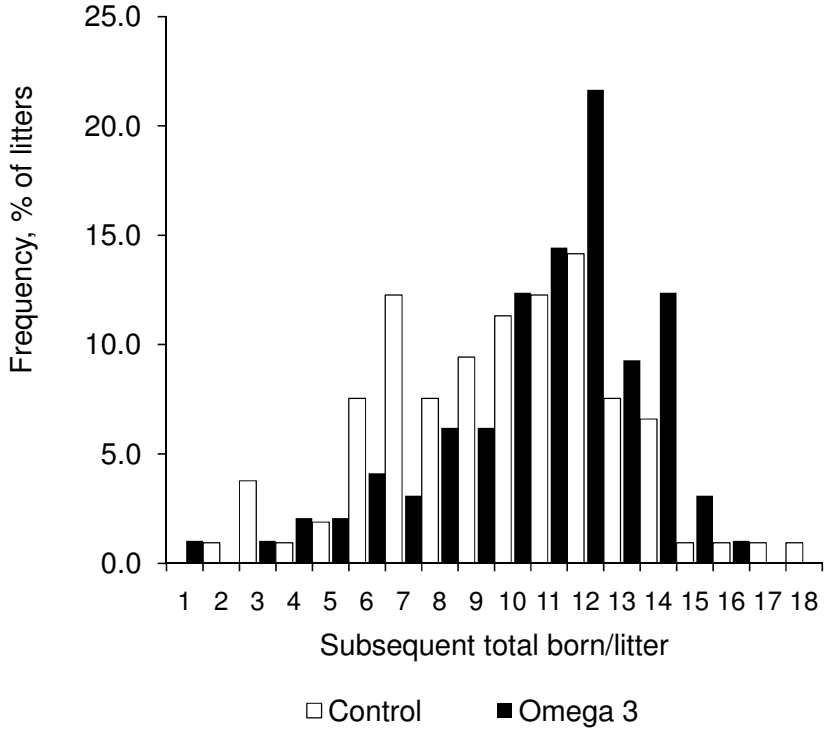

Figure 3.1 The frequency of litter size total born in the parity subsequent to sows being fed a control diet (Control) or a diet containing 3 g of fish oil/kg of diet (Omega-3) in the previous parity for a total of 27 d from 8 d prefarrowing continuing in lactation until weaning at 18.7 ± 0.1 d

3.4 Discussion

The present study was undertaken to determine if adding omega-3 PUFA by partially replacing tallow with fish oil in the lactation diet increases subsequent reproductive performance in sows. The results from this study demonstrated that feeding sows with 3 g fish oil/kg of diet prefarrowing for 8 d and continuing through lactation for 19 d to weaning, a total duration of 27 d, significantly increased subsequent total litter size born and born live by 1 pig per litter. Although litter size was increased, there was no effect on pregnancy maintenance or farrowing rate. This is the first time to our knowledge that an increase in sow reproductive performance to dietary omega-3 PUFA from fish oil in the lactation diet fed prefarrowing and during lactation in the previous parity has been reported. The dietary response on subsequent litter size born to omega-3 supplementation in the present study may have been due to the parity range of sows used, as this has not been observed by others using gilts (Estienne et al., 2006). Support for this suggestion comes from previous work showing embryo survival is decreased in sows of higher parity compared to gilts and parity 1 sows (Foxcroft et al., 2006).

There was no increase in feed intake during lactation in the Omega-3 sows, suggesting that the effect of the omega-3 fatty acids on subsequent reproduction is independent of energy intake. The energy level of the Control and Omega-3 diet was formulated to be of equal value to remove the possibility of a response due to dietary energy. Adding fat to diets can lead to oxidation and a reduced appetite (Close and Cole, 2000a), however sources of good quality tallow and fish oil and the addition of antioxidants were used in this experiment. Vitamin E as the anti-oxidant, α-tocopherol, was added at an equivalent of 179 I.U. in both the Control and Omega-3 diet which was well above the minimum requirement (NRC, 1998). Others have reported no adverse effect of tallow or animal fat up to 10% inclusion in the diet on lactation feed intake (Johnston et al., 1986; Tilton et

al., 1999). As there was no difference in sow feed intake we conclude that the observed response on subsequent litter size is due to the nature of the fat source, rather than an energy response.

There was no immediate effect of feeding the omega-3 PUFA diet for 8 d prefarrowing and during lactation on the viability of piglets, feed intake of sows, piglet weaning weight or the number of piglets weaned in the litter. The lack of a response in birth weight due to omega-3 fatty acids prefarrowing is similar to the findings of Rooke et al. (2001b). In contrast, increases in neonatal growth and weaning weight have been observed when high dietary levels (3 to 4% of diet) of fish oil were used in diets fed to sows during the last trimester (Rooke et al., 2001c). Lower daily intakes during the feed restriction period prefarrowing as well as a short duration of omega-3 dietary feeding prior to farrowing in the present study may explain the lack of a response on birth weight and preweaning growth rate.

Palmer et al. (1970) fed mated gilts a diet containing 6% whole fish meal continuously over two parities commencing in gestation and reported an increase in litter size by 0.5 and 1.2 pigs born live in parities 1 and 2, respectively, compared with controls. They also reported an increase in farrowing rate of 8% in parity 2 in sows fed diets containing fish meal. Unlike the dietary formulations used in the present study, Palmer et al. (1970) did not use fish oil, and the reproductive outcomes may have been a consequence of the dietary supply of other nutrients, such as the antioxidants vitamin E and selenium as suggested by Baker et al. (1974) who reported no response to fish meal in sows. In their abstract, Webel et al. (2004) reported an increase in subsequent litter size by 0.8 pig when sows were fed a PUFA supplement sourced from fish oil during 8 d prefarrowing, lactation (16 d) and postweaning to mating. In contrast, others have failed to demonstrate any benefits to reproductive performance in gilts (Estienne et al., 2006) and sows (Perez-Rigau et al., 1995) when fed a diet with elevated omega-3 PUFA from fish oil.

The reasons for these differences may relate to the duration or timing of omega-3 supplementation. Webel et al. (2004) reported that supplementation for 8 d before farrowing and continuing to mating increased the subsequent litter size born compared to sows supplemented from farrowing until mating. In our current experiment, supplementation did not extend beyond weaning and yet a similar response in subsequent litter size was seen. Therefore, the need to continue feeding diets with omega PUFA after weaning may not be necessary. However, it seems that supplementation during lactation is necessary. For example, Perez-Rigau et al. (1995) did not observe a significant difference in the total number of foetuses when sows were fed diets containing 40 g menhaden oil/kg of diet from d 1 postweaning until slaughter at 42 d of gestation.

The mechanism for increased litter size in sows fed diets containing omega-3 PUFA from fish oil as observed in this study remains to be determined. When gilts were fed diets containing the omega-3 fatty acids EPA and DHA from fish oil, the levels of these PUFA increased in embryos and the endometrium (Perez-Rigau et al., 1995; Brazle et al., 2009). In their experiments, dietary treatments continued during early pregnancy until slaughter at six weeks of gestation. In the present study, dietary supplementation ended at weaning so the response we observed on subsequent litter size born is more likely to be due to improvements in follicular development or oocyte quality. In the pig, oocyte quality influences embryonic development and survival (Ashworth and Antipatis, 1999; Quesnel et al., 2010) and in turn, litter size. Hence feeding omega-3 PUFA enriched diets during lactation when those oocytes that ovulate are undergoing final growth and maturation may explain the response in litter size. However specific effects of omega-3 PUFA on oocyte quality and embryo survival in sows have not been reported.

In conclusion, it is demonstrated for the first time that feeding sows a diet containing omega-3 fatty acids from 3 g fish oil/kg of diet from 8 d before farrowing and continuing through lactation for a further 19 d until weaning increased total born and live born in the subsequent parity with no change

in farrowing rate. This response was independent of total energy intake and possibly influences oocyte quality and embryo survival.

Chapter 4 Effect of omega-3 supplementation on reproductive performance in gilts. I. Effect of duration

4.1 Introduction

Gilt farrowing rate and litter size born are important traits that determine a sows' lifetime production, with poor reproductive performance reported as the major cause for the early culling of sows (Hughes and Varley, 2003). In the previous chapter, we reported that feeding sows a diet supplemented with omega-3 PUFA from 3 g fish oil/kg of diet for 8 d prefarrowing and continued through lactation for 19 d until weaning increased litter size in the subsequent parity by 0.9 live born per litter.

There are few studies published on the reproductive response of prepubertal gilts supplemented with omega-3 PUFA. Palmer et al. (1970) reported an increased pregnancy rate and parity 1 litter size when mated gilts were fed diets containing fishmeal from mating and continued through gestation. Perez-Rigau et al. (1995) compared the ovulation and embryo survival of gilts fed diets for 50 d prior to mating with added oil from coconut (high in saturated fat), soybean oil (high in omega-6 PUFA), menhaden fish oil (high in omega-3 PUFA), and corn starch. Although there was no difference between dietary fat sources on embryo survival in the first gilt trial, in a second trial embryo survival was increased by 19% in gilts fed menhaden oil (Perez-Rigau et al., 1995).

In Chapter 3, we reported an increase in litter size born subsequent to multiparous sows being fed omega-3 supplemented diets prefarrowing and during lactation, a duration of 27 days, which was a considerably shorter supplementation period to that described by others in multiparous sows (Palmer et al., 1970; Webel et al., 2004) and gilts (Perez-Rigau et al., 1995; Estienne et al., 2006). There is a

paucity of published studies where omega-3 PUFA supplementation from fish oil have been investigated in gilts and sows, whilst the methodology varies considerably. The duration and level of fish oil supplementation have been identified as possible factors influencing the reproductive response to omega-3 PUFA in pigs (Webel et al., 2004; Estienne et al., 2006). The aim of the present study and subsequent study therefore was to examine the effect of duration and dose of omega-3 PUFA supplementation on gilt reproductive performance. In the present study, we examined whether dietary supplementation with fish oil fed for three weeks prior to mating is sufficient to improve litter size at first parity when compared with gilts fed supplemented diets for six weeks.

4.2 Materials and Methods

4.2.1 Animals and diets

Over seven non-consecutive weeks commencing in winter, 570 Large White and Landrace gilts were selected for the experiment at 169.8 ± 0.1 d of age (mean ± SE). Within breed, gilts were randomized by age and live weight and allocated to one of three dietary treatment feeding regimens fed *ad libitum* until mating: gilts fed the Control diet fed from 24 weeks to mating (Control); or gilts fed the Omega-3 diet from 24 weeks of age to mating (Omega-3 for 6 weeks); or gilts fed the Control diet from 24 weeks to 27 weeks of age followed by the Omega-3 diet to mating (Omega-3 for 3 weeks). The Control diet contained no added fish oil and was based on the cereal grains wheat, wheat by-products, and tallow and provided 14 MJ DE/kg, 142 g crude protein/kg, 0.53 g available lysine/MJ DE (Table 4.1). The same base diet was formulated with supplemental omega-3 fatty acids (Omega-3) supplied as 3 g fish oil/kg of diet as a partial substitute of tallow (Table 4.1). Diet manufacture and ingredient suppliers of tallow and fish oil are detailed in Chapter 2.

4.2.2 Feeding and animal management

Gilt live weight and backfat depth at the P2 site (Close and Cole, 2000a) were measured at 24 weeks of age prior to treatment allocation. Every three weeks, approximately 80 pre-pubertal gilts were transported to the commercial piggery and housed on partially concrete slatted floors in groups of 12 – 14 gilts according to treatment at 1.6 m^2/gilt. Feeders in the pens of gilts that were allocated to Omega-3 for 3 weeks were emptied of the unsupplemented Control diet before filling with the Omega-3 diet at 27 weeks of age. *Ad libitum* feed intake between 24 weeks of age and mating was not recorded due to the limitations of the commercial feeding system.

Mature boars were used daily to stimulate oestrus in gilts commencing at 24 weeks of age until mating. A sample of gilts (6 per treatment) had blood sampled in a repeatable measure sample procedure by jugular venipuncture at 0, 3, 7 and 21 d from the start of the experiment (24 weeks of age). Blood samples were collected on ice and plasma stored at -20^oC until being analysed for fatty acids at the Nutrition and Functional Foods Laboratory, The University of Adelaide, Waite Campus, SA. Refer to Chapter 2 for fatty acid analysis methodology.

At six weeks from the start of feeding, or on their second oestrus if it was observed before six weeks on trial, gilts were moved to mating pens (1.4 m^2/gilt) in the same shed and continued on their treatment diet until mating. All gilts were mated by artificial insemination in an am/pm/am service in the presence of a mature boar. Following their last service, half the gilts were weighed and measured for backfat P2 as a subset. The maximum time for keeping non-cycling gilts on trial before culling was set at 15 weeks (39 weeks of age) after treatment allocation due to the restriction of space in the piggery.

From mating, all gilts were housed in individual gestating stalls for six weeks then housed in groups of two to three animals (1.4 m^2/gilt) until 112 d gestation before entry to a commercial farrowing room prior to the birth of their first litter. From the day after the last AI service, all gilts were given a daily

allowance of 2.4 kg of a commercial gestating sow diet (13 MJ DE; 146 g crude protein/kg; 5.9 g total lysine/kg; Table 4.1), increasing to 2.7 kg after 13 weeks of pregnancy until entering the farrowing house. Thereafter, gilts were fed 3.0 kg once a day of a commercial lactation diet (15.0 MJ DE/kg; 176 g crude protein; 0.51 g available lysine/MJ DE; Table 4.1). Mated sows were checked for a return to oestrous using a mature boar and applying back pressure to the sow and were tested for pregnancy at d 30. Reasons for sow removal, subsequent farrowing rate, and subsequent litter size born were recorded within 24 h of birth. Total litter size is defined as the sum of the number of piglets born live, still born and mummified foetuses.

4.2.3 Fatty acid analysis

Stored samples of experimental diets (approximately 200 g) and plasma (5 mL) were analyzed at the Nutrition and Functional Foods Laboratory, The University of Adelaide, Waite Campus, SA. A full description of diet and plasma fatty acid analyses is provided in Chapter 2. The fatty acid profile of the treatment diets in g/100 g of total fatty acids extracted is summarized in Table 4.2. The fatty acid levels in plasma at 0, 3, 7 and 21 d for gilts on Control and Omega-3 diets are illustrated in Figure 4.1.

4.2.4 Statistical analysis

Data was analysed using SPSS v 18.0 with breed line as a random factor. Block was also included as a random factor in the General Linear Model (GLM) Univariate ANOVA. Age, live weight and back fat at the start of the experiment were included in the GLM ANOVA model if identified to have significant effects. The proportion of gilts with single or multiple oestrus at first mating, the proportion of gilts mated of the commencement experimental population, and the proportion of gilts farrowing from their first mating event were analysed by chi square. Pairwise comparisons were performed using Least Significant Difference. Mean values are expressed as mean ± standard error (SE). Fatty acid samples were analysed by GLM Univariate Model ANOVA using repeated measures.

Probability values $P < 0.05$ were described as significant, and $P < 0.10$ were used to describe trends. Data was analysed for 563 gilts of the 570 allocated to the study. Seven animals had incomplete data records and were excluded.

4.3 Results

4.3.1 Plasma fatty acid response to supplementation

The dietary fatty acid profile in Table 4.2 shows that 3 g fish /kg diet resulted in a nine-fold increase of EPA and DHA in the Omega-3 diet compared with the Control diet. Plasma fatty acids EPA and DHA were increased in gilts fed the Omega-3 diet after three days of supplementation (Figure 4.1). This continued to increase so that by d 21, EPA and DHA had increased by 2.7 fold ($P < 0.001$). The difference between treatments in plasma levels of fatty acids was therefore lower than that supplied in the diet, but was still highly significant. The level of plasma LIN and ALA was unaffected by dietary treatment (Figure 4.1). The omega-6 long-chain fatty acid, ARA was higher ($P < 0.05$) in plasma from gilts fed the Omega-3 diet on d 0 and on d 3 (Figure 4.1). However by d 7, there was no significant effect of supplementation with fish oil on ARA concentration in the plasma by d 21. The number of days gilts were fed the supplemented diet averaged 23.7 ± 1.3 d for gilts on Omega-3 for 3 weeks and 46.9 ± 1.3 d by gilts fed Omega-3 for 6 weeks ($P < 0.001$).

4.3.2 Treatment effect on live weight and fatness

Gilts commenced the experiment at 169.8 ± 0.1 d of age with a similar body weight (108.0 ± 0.4 kg) and backfat P2 (11.8 ± 0.1 mm). At mating, a subset of animals from each treatment was weighed and backfat P2 recorded (Table 4.3). There was no difference in live weight, backfat P2 at mating, nor daily weight gain or P2 gain between gilts on the Control and Omega-3 groups.

4.3.3 Onset of oestrus and reproductive performance

The proportion of gilts recorded in oestrus and the number of days taken to respond to boar stimulation is summarised in Table 4.4. There were several gilts that recorded an initial oestrus, but failed to be detected in oestrus again within the maximum period before culling at 39 weeks of age. Within this category, there tended to be more gilts from the group fed the Omega-3 diet for 6 weeks (4 x Control; 8 x Omega-3 for 6 weeks; 1 x Omega-3 for 3 weeks; χ^2 5.56; $P = 0.06$).

The proportion of animals removed from the herd was attributed to reproductive and physical causes. Across all treatments, there were 108 out of the 563 gilts (19.2%) culled prior to mating. Poor locomotion (n = 44) described 7.8% of the allocated gilts, whilst 8 gilts (1.4% of allocated) were culled for poor body condition (condition score of < 2) prior to mating. Across all treatments, 56 gilts (9.9%) were classed as anoestrous, however as mentioned above, 13 of these (2.3% of allocated) were recorded to have displayed an initial pubertal oestrus but were not detected in oestrus again (failed to re-cycle or silent oestrus). There were no differences between dietary treatments on gilt cycling or gilt removal from the breeding herd prior to mating (Table 4.4). There was a significant treatment difference on age at mating with gilts on Omega-3 for 6 weeks being mated later ($P < 0.05$), although there was no treatment difference in the days to puberty onset from the time of allocation (Table 4.4).

The majority of gilts were mated on their second oestrus. Of the Controls, 120 of the 154 gilts mated (77.9%) occurred on 2nd or 3rd detected oestrus compared to 109 (74.1%) gilts on the Omega-3 diet for 6 weeks and 112 (72.7%) gilts on the Omega-3 diet for 3 weeks. There was no significant difference in the proportion of gilts mated on 2nd and/or 3rd oestrus among treatments (χ^2 1.18; $P = 0.554$).

One gilt (Control) had a total litter of one piglet (still born) and was excluded from the litter data analysis as atypical. There was no significant difference in the number of piglets born between

Controls and supplemented gilts as either Omega-3 for 6 weeks or Omega-3 for 3 weeks (Table 4.5). Although the data was collected from gilts mated from July to December, the effect of season, measured as mating week, on farrowing rate (P = 0.846) and litter size (P = 0.070) was not significant. The interaction of season x treatment on litter size born was not significant (P = 0.690). The main effects of breed on litter size (P = 0.393) and treatment x breed interaction (P = 0.356) were also not significant.

Table 4.1 Ingredient and calculated nutritional composition of experimental diets (g/kg as fed basis) fed to gilts allocated to either a control diet (Control) or a diet containing omega-3 PUFA from 3 g fish oil/kg of diet (Omega-3) until mating[1]

	Treatment diet		Commercial diet	
Ingredient, g/kg	Control	Omega-3	Gestation	Lactation
Wheat	714	714	500	488
Barley	-	-	246	97
Millrun (wheat middlings)	174	174	190	80
Soybean meal	-	-	-	90
Canola meal	-	-	-	55
Meat meal	52	52	16	72
Bloodmeal	-	-	-	20
Tallow[2]	30	27	10	62
Molasses	-	-	-	10.7
Salt	2.0	2.0	3.0	3.5
Limestone	10.1	10.1	12.3	8.0
Water	10.0	10.0	10.0	-
Di-calcium phosphorus	-	-	9.0	-
Potassium chloride	-	-	-	4.2
Fish oil[3]	-	3.0	-	3.0
Synthetic lysine (L-lysine HCl)	3.8	3.8	1.5	1.8
Synthetic methionine (DL-methionine)	0.5	0.5	-	0.6
Synthetic threonine	1.4	1.4	0.1	0.8
Mineral vitamin premix[4]	2.4	2.4	2.3	2.3
Phytase	0.1	0.1	0.1	0.1
Xylanase	0.2	0.2	-	0.3
Monensin	1.0	1.0	1.0	1.0
Calculated nutrient analyses, g/kg				
Digestible energy (MJ/kg)	14.0	14.0	13.0	14.9
Crude protein	169.1	168.9	145.6	215.8
Crude fat[5]	49.0	49.0	34.1	90.2
Crude fibre	34.0	34.0	42.0	35.9
Calcium	8.7	8.7	9.0	9.0
Total phosphorus	5.7	5.7	6.4	5.9
Available phosphorus	4.3	4.3	4.8	4.7
Lysine	8.7	8.7	5.9	11.9
Ileal digestible lysine (g/MJ DE)	0.54	0.54	0.39	0.68
Methionine	3.0	3.0	2.2	4.0
Methionine + cystine	6.2	6.2	5.7	7.7
Threonine	6.3	6.3	4.4	8.3
Valine	7.2	7.2	5.7	10.3
Isoleucine	5.3	5.3	4.1	7.0
Tryptophan	1.7	1.7	1.8	2.4

[1]Treatment diets commenced at 169.8 ± 0.1 d of age and were fed *ad libitum* until mating. The commercial gestation diet was fed 2.4 kg/d until 13 weeks of age, 2.7 kg/d until prefarrowing at 112 d of gestation, followed by 3 kg/d of lactation diet.
[2]Tallow supplied by Biodiesel Producers, Barnawartha, VIC.
[3]Fish oil supplied by Feedworks, Sunbury, VIC.
[4]Premix provided the following nutrients (per kg air-dry diet): copper, 20 mg; iron, 80 mg; organic iron, 75 mg; manganese, 40 mg; zinc, 100 mg; iodine, 1 mg; selenium inorganic, 0.15 mg; selenium organic, 0.15 mg; chromium picolinate, 3.2 mg; betaine, 100 mg; antioxidant (Endox), 100 mg; vitamin A (retinol), 15,000 I.U.; vitamin D (cholecalciferol), 1,500 I.U.; vitamin E (α-tocopherol), 120 mg; vitamin B_2 (riboflavin), 3.5 mg; vitamin B_6 (pyridoxine), 2 mg; vitamin B_{12} (cyanocobalamin), 0.02 mg; biotin, 0.2 mg; folic acid, 0.5 mg; niacin, 20 mg; pantothenic acid, 5 mg.
[5]Analyzed crude fat levels were 55.6 g/kg (Control) and 57.9 g/kg (Omega-3).

Table 4.2 Fatty acid composition of experimental diets (g/100 g total fatty acid as fed basis) fed to gilts allocated to either a control diet (Control) or a diet containing omega-3 PUFA from 3 g fish oil/kg of diet (Omega-3) until mating[1]

	Treatment diet	
Fatty acid (Common name)	Control	Omega-3
C16:0 (palmitic acid)	20.6	20.1
C16:1 (palmitoleic acid)	1.91	2.05
C18:0 (stearic acid)	11.7	10.8
C18:1 (oleic acid)	28.8	28.5
C18:2 n-6 (α-linoleic acid)	29.3	29.7
C18:3 n-6 (γ-linolenic acid)	0.03	0.05
C18:3 n-3 (α-linolenic)	1.86	1.85
C20:4 n-6 (arachidonic acid)	0.19	0.18
C20:5 n-3 (eicosapentaenoic acid)	0.03	0.27
C22:5 n-3 (docosapentaenoic acid)	0.09	0.19
C22:6 n-3 (docosahexaenoic acid)	0.04	0.35
Total n-6	29.7	30.2
Total n-3	2.37	2.99
n-6:n3 ratio	12.5	10.1
Total saturated	35.7	34.2
Total transaturated	0.32	0.33

[1]Treatment diets commenced at 169.8 ± 0.1 d of age and were fed *ad libitum* until mating. The commercial gestation diet was fed 2.4 kg/d until 13 weeks of age, 2.7 kg/d until prefarrowing at 112 d of gestation, followed by 3 kg/d of lactation diet.

Table 4.3 Live weight and backfat P2 and weight and P2 gain (mean ± SE) on the subset of gilts between 24 weeks of age and mating fed Control or Omega-3 diets for either 6 weeks or 3 weeks prior to mating[1]

	Treatment			
	Control	Omega-3		
Item[2]	6 weeks	6 weeks	3 weeks	*P* value
Number gilts assessed	98	92	101	
Live weight, kg				
24 weeks of age	108.6 ± 1.0	108.5 ± 1.1	106.0 ± 1.0	0.140
mating	145.1 ± 1.4	145.2 ± 1.6	143.2 ± 1.4	0.528
daily gain, g/d	0.936 ± 0.022	0.925 ± 0.022	0.939 ± 0.022	0.907
Backfat P2, mm				
24 weeks of age	11.8 ± 0.3	12.1 ± 0.3	11.8 ± 0.3	0.656
mating	14.7 ± 0.4	15.0 ± 0.4	14.5 ± 0.3	0.633
daily fat gain, mm/d	0.073 ± 0.009	0.065 ± 0.010	0.068 ± 0.009	0.832

[1]Gilts were fed Control or Omega-3 diets for 6 weeks or 3 weeks *ad libitum* from 169.8 ± 0.1 d of age to mating at 215.5 ± 0.7 d of age.
[2]Estimated marginal mean values with age at treatment allocation (169 ± 0.1 d days of age) and age at mating (215.5 ± 0.7 d of age) included as covariate factors.

Table 4.4 Puberty response and age at mating (mean ± SE) of gilts fed Control diets or Omega-3 diets containing 3 g fish oil/kg of diet fed for either 6 weeks or 3 weeks prior to mating[1]

	Treatment			
	Control	Omega-3		
Item	6 weeks	6 weeks	3 weeks	*P* value
Number gilts allocated	187	186	190	
Number gilts cycled[2]	159 (0.85)	159 (0.85)	160 (0.84)	χ^2 0.12, 0.941
Number anoestrous gilts.[2]	14 (0.075)	15 (0.080)	14 (0.074)	χ^2 0.07, 0.964
Total number culled pre-mating[2,3]	33 (0.18)	39 (0.21)	36 (0.19)	χ^2 0.67, 0.714
Onset of oestrus, d[4]	25.7 ± 1.8	29.4 ± 1.7	27.7 ± 1.8	0.325
Age at mating, d[4]	213.1 ± 1.2[a]	217.7 ± 1.3[b]	214.6 ±1.3[ab]	0.033

[1]Gilts were fed Control or Omega-3 diets for 6 weeks or 3 weeks *ad libitum* from 169.8 ± 0.1 d of age to mating at 215.5 ± 0.7 d of age.
[2]Proportion of total allocated at the start of the study in parenthesis ().
[3]Includes gilts culled for locomotion and other physical causes and those culled as 'anoestrous' that showed a Standing Heat Response initially and failed to display oestrus after first detection.
[4]Estimated marginal mean values with 24 week weight (108.3 kg) and 24 week P2 (11.8 mm) included as covariate factors.
[ab]Estimated marginal mean values within row with different superscripts are significant ($P < 0.05$).

Table 4.5 Farrowing rates (sows successfully farrowed from 1st mating) and first parity litter size (mean ± SE) following feeding of Control diets or Omega-3 diets containing 3 g fish oil/kg of diet fed for either 6 weeks or 3 weeks prior to mating[1]

	Treatment			
	Control	Omega-3		
Item	6 weeks	6 weeks	3 weeks	*P* value
Number gilts mated	154	147	154	
Number sows farrowed	130	128	136	
Farrow rate, %	84.4	87.1	88.3	χ^2 1.05, 0.592
Parity 1				
Live born, number pigs	10.6 ± 0.2	11.0 ± 0.2	10.6 ± 0.2	0.283
Still born, number pigs[2]	1.1 ± 0.1	0.9 ± 0.1	0.9 ± 0.1	0.459
Total born, number pigs[2]	11.6 ± 0.2	11.9 ± 0.2	11.6 ± 0.2	0.465

[1]Gilts were fed Control or Omega-3 diets for 6 weeks or 3 weeks *ad libitum* from 169.8 ± 0.1 d of age to mating at 215.5 ± 0.7 d of age.
[2]Includes piglets still born and mummied foetuses.

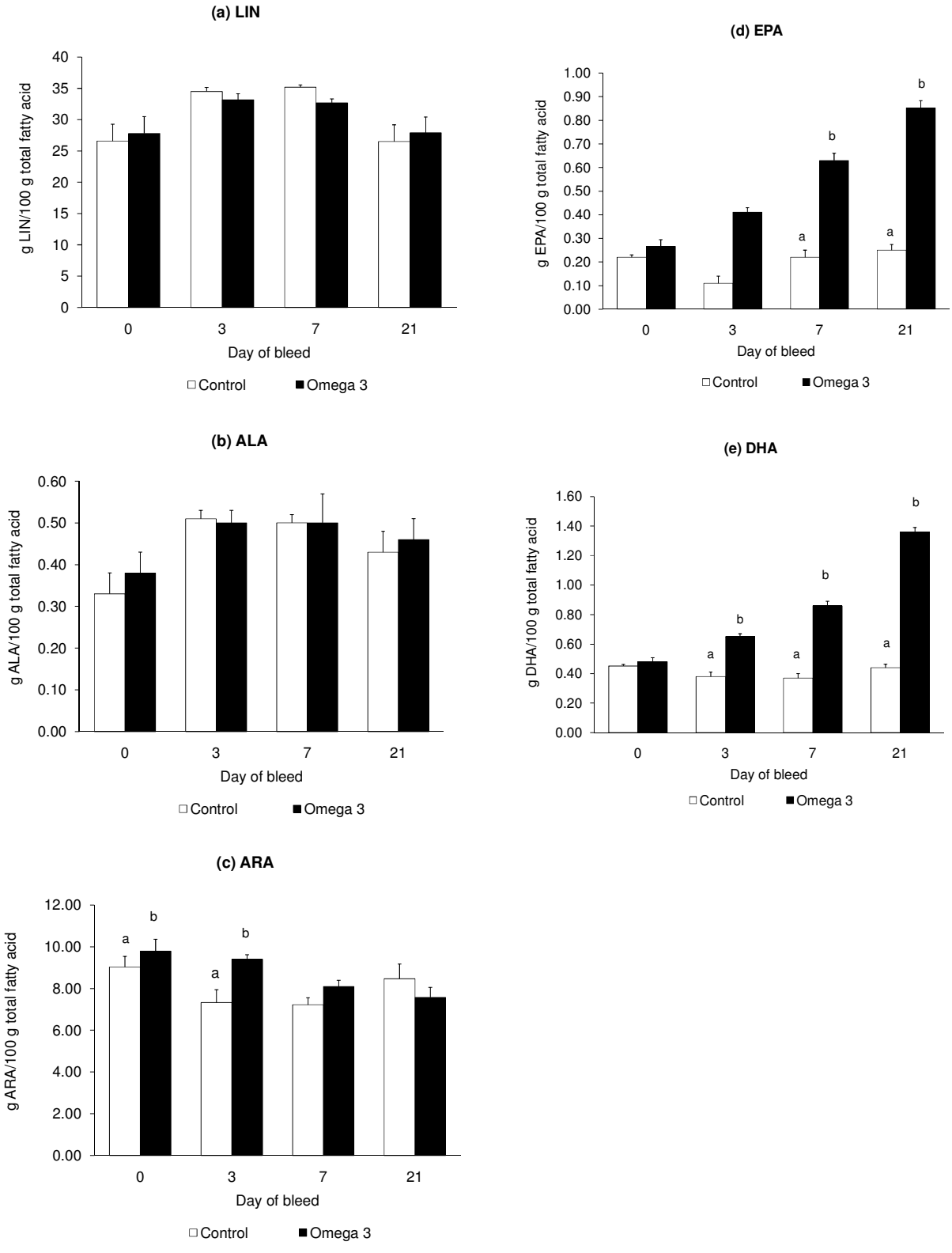

Figure 4.1 Mean ± SE plasma fatty acids in gilts (n = 6 per treatment in a repeated measures sampling procedure) at 0, 3, 7 and 21 d from the commencement of feeding either the Omega-3 or unsupplemented Control diet fed *ad libitum* between 24 and 27 weeks of age

[ab]Mean values within day of bleeding differ significantly ($P < 0.01$)

4.4 Discussion

The aim of the present study was to determine the reproductive response of gilts fed a diet supplemented with omega-3 PUFA during puberty until mating, and secondly to determine if the duration of feeding prior to mating affected this response. Gilts fed the Omega-3 diet for six weeks prior to mating had a similar farrowing rate and litter size born as Control gilts. In addressing our second aim, supplementation for three weeks prior to mating produced a similar reproductive outcome compared with longer term feeding. From these results, increasing the duration of omega-3 supplementation did not improve the reproductive response to omega-3 PUFA.

The response to dietary omega-3 fatty acid supplementation in the pubertal gilt has not been widely studied. Palmer et al. (1970) reported an increase in gilt farrowing rate and parity 1 litter size born when whole fish meal was added to the diet fed for 30 d prior to mating and continued throughout gestation. Using corn-soybean based diets, Spencer et al. (2004) briefly reported that litter size born in parity 1 was increased when a protected omega-3 PUFA product based on fish oil was fed as a dietary supplement (1.5% of the diet) for a minimum of 30 d prior to mating in gilts. In the present study, there was no effect of omega-3 supplementation when fed for a similar period (6 weeks) prior to mating on reproductive performance, except for an increased age at mating. Gilt farrowing rate and litter size born in the first parity were unaffected by the diet and also by duration of omega-3 supplementation. Estienne et al. (2006) was also unable to demonstrate a reproductive response to omega-3 supplementation from fish oil in gilts, however they suggested their use of altronogest to synchronize oestrus for mating may have improved embryo survival and baseline fertility and overridden any benefit from omega-3 supplementation (Estienne et al., 2006).

Live weight and fat deposition was unaffected by dietary treatment. In order to evaluate the response to specific fatty acids, it was necessary to formulate both the Control and Omega-3 diet to contain the same level of dietary energy. As there was no difference in weight change or P2 backfat

over the period of supplementation in the current study, we can infer that the partial replacement of tallow with fish oil at low levels resulted in no change in dietary energy supply. Although *ad libitum* feed intake was not measured between the start of feeding and mating, it would appear from the weight and P2 data that intake was unaffected by 3 g fish oil/kg diet in pubertal gilts when formulated to be isocaloric with an unsupplemented diet.

There was a significant increase in the age at mating in gilts fed the Omega-3 diet for 6 weeks compared to gilts fed the Control diet. These results are in contrast to those reported by Estienne et al. (2006) and Perez-Rigau et al. (1995) who observed no effect of fish oil diets on age at puberty or the onset of puberty, although in the case of Estienne et al., the use of altronogest to synchronize oestrus would have confounded the natural oestrous cycle. In the present study, diet had no effect on the proportion of gilts that exhibited oestrus nor the number culled for failing to be mated by 39 weeks of age. Therefore omega-3 may alter the timing of oestrus but does not appear to affect the establishment of the gilts natural oestrous cycle.

Dietary supplementation of cereal – tallow based diets with fish oil was shown to be highly effective at increasing the concentration of the long-chain omega-3 fatty acids EPA and DHA in plasma, supporting an earlier dietary dose response study by Smits et al. (2007). In the present study, we found that the uptake of fatty acids occurs rapidly, with elevated plasma being significant after only 3 d from diet introduction. Plasma omega-3 PUFA continued to increase over the 21 days of assessment. As we did not measure plasma PUFA beyond this, we cannot conclude if plasma EPA and DHA concentration was maximized by 21 d after diet introduction. The addition of a high quality fish oil product was shown to be effective as a means to increase the plasma EPA and DHA without affecting the level of the omega-3 fatty acid precursor, ALA, or the omega-6 precursor, LIN. The rate of biosynthesis of long-chain PUFA from LIN and ALA is regarded as low (Rooke et al., 2000; Azain, 2004), so the majority of EPA and DHA in the plasma would be directly due to dietary supply from

the fish oil. It is clear from our data that supplementation of a wheat-based diet with low levels of fish oil was enough to substantially increase EPA and DHA in plasma by nearly three-fold compared to Control gilts fed unsupplemented diets. Although there was a higher ARA content in plasma by d 3 in gilts fed the Omega-3 diet, there was no difference in this omega-6 fatty acid by d 7 and thereafter. The high level of plasma ARA in Omega-3 gilts at d 0 and d 3 is likely due to an unknown factor influencing the level of this fatty acid before the experiment commenced. The concentration of ARA in plasma is important as it is the substrate for the cyclooxygenase enzyme complexes (COX-1 and COX-2) during prostaglandin synthesis (Caughey et al., 2005). At high levels of fish oil (> 6 g/kg diet), ARA can be reduced in plasma (Smits et al., 2007), however the results from this experiment confirm that ARA was not affected by low levels of fish oil.

In the present study, we followed the dietary regimen described by Spencer et al. (2004) and did not continue feeding the Omega-3 diet after mating and during gestation. The shorter feeding duration of three weeks prior to mating was sufficient to increase plasma omega-3 fatty acids, but continuing to feed the Omega-3 diet after mating, as practiced by Palmer et al. (1970) and Perez-Rigau et al. (1995), may be necessary to elicit a reproductive response. A higher level of supplementation, as proposed by Estienne et al. (2006), may also be necessary. The level of fish oil used in the Omega-3 diet in the present study was based on our results from Chapter 3, however feed intake in pubertal gilts is likely to have been lower than we recorded in lactating multiparous sows (7.9 kg/d).

In conclusion, there was no effect on the attainment of puberty, the proportion of oestrous gilts, farrowing rate or parity 1 litter size born when a diet supplemented with omega-3 PUFA by the addition of 3 g fish oil/kg of diet was fed to gilts *ad libitum* for either three or six weeks prior to mating compared to gilts fed an unsupplemented diet. Feeding the supplemented diet for three weeks prior to mating provided sufficient time to allow an increase in plasma omega-3 EPA and DHA, however continuing to feed the Omega-3 diet in gestation may be necessary to invoke a reproductive

response in gilts. This formed the rationale for investigating a dose response in gilts fed supplemented omega-3 diets before mating and continuing through gestation in the next chapter.

Chapter 5 Effect of omega-3 supplementation on reproductive performance in gilts. II. Effect of dose

5.1 Introduction

In Chapter 3 sows fed a diet containing omega-3 PUFA from 3 g fish oil/kg of diet for 8 d prefarrowing and continued through lactation until weaning produced more piglets born in the subsequent parity. However in Chapter 4, feeding the same level of fish oil to gilts prior to their first mating did not increase farrowing rate or the number of piglets born in the first litter. Perez-Rigau et al. (1995) reported in one of two trials a trend towards a higher embryo survival when gilts were fed diets supplemented with 4% fish oil compared to vegetable oils or starch. In comparison to the feeding regimen used in Chapter 4, Perez-Rigau et al. fed the supplemented diets for 50 d pre-mating and continued through gestation until slaughter at six weeks (Perez-Rigau et al., 1995), suggesting that continuing supplementation during early pregnancy may be necessary to increase litter size in gilts. However, Estienne et al. (2006) observed no difference in ovulation rate or embryo survival when gilts were fed a dietary supplement of long-chain omega-3 PUFA prior to mating and during gestation and suggested that the level of supplementation may have been insufficient to improve reproduction in highly fertile gilts.

In the present study, a similar experimental feeding regimen as described by Perez-Rigau (1995) was adopted. Furthermore, the study was designed to provide an insight into whether the increase in litter size recorded in Chapter 3 in omega-3 supplemented sows was possibly a result of increased ovulation rate or embryo survival. The aim of this study therefore was to examine the reproductive performance of gilts fed diets containing fish oil at either 3 or 10 g fish oil/kg diet when fed from 24

weeks of age up to mating and continued through to early pregnancy compared to gilts fed unsupplemented diets.

5.2 Materials and Methods

5.2.1 Animals and diets

Over nine months commencing in summer, a total of 356 Large White x Landrace F1 cross gilts in total were allocated across dietary treatment every six weeks to the study at 24 weeks of age. From 24 weeks of age, gilts were allocated to either a diet which contained tallow and no fish oil (Control); a diet where tallow was partially replaced with omega-3 from fish oil at a rate of 3 g fish oil/kg of diet (Omega-3, 3 g/kg); or a diet where tallow was partially replaced with 10 g fish oil/kg of diet (Omega-3, 10 g/kg). Allocation of gilts to dietary treatment was randomised for live weight at the start of the study. The Control and Omega-3 diets were similar in ingredient composition to the Control and Omega-3 diets used in Chapter 4, with a change in nutrient composition due to new season grains (Table 5.1). Each diet was isocaloric and provided 14.0 MJ DE/kg; 155 g crude protein/kg; 0.52 g available lysine/MJ DE. Diet manufacture and ingredient suppliers of tallow and fish oil are detailed in Chapter 2.

5.2.2 Feeding and animal management

At 17 weeks of age, gilts were housed in groups of eight (0.83 m^2/pig) in pens with partially slatted concrete floors. All gilts were offered the Control diet (Table 5.1) for the period of pre-trial acclimatisation. At 24 weeks of age, gilts were individually weighed and backfat P2 was measured. A sub-sample of 102 gilts were bled by jugular venipuncture the day before treatment feeding commenced at 24 weeks of age. A detailed description of blood sampling procedure is provided in Chapter 2. At treatment allocation, animals were moved to gilt pens of 6.65 m^2 in groups of four to six according to dietary treatment. Gilts were fed their treatment diet from 24 weeks of age *ad libitum* until mating.

Five weeks later at 29 weeks of age, gilts were moved to an adjacent mating shed and housed within treatment in groups of six to eight in pens of 14.4 m^2 and continued on their treatment diet *ad libitum.* A delayed boar stimulation program commenced at 29 weeks of age to avoid any non-dietary treatment variation on the onset of oestrus and age at mating. Gilts were given 15 min of boar stimulation each day in detection mating pens in front of several boars. On the first observed oestrus after 29 weeks of age, gilts were mated by artificial insemination in an am/pm/am service in the presence of a boar. Gilts that did not display a standing heat response within 35 d of entering the mating shed (within 36 weeks of age) were defined as anoestrous. Other reasons for gilt wastage such as culling for locomotion and mortality were also recorded.

The day after mating, gilts were weighed and backfat P2 measured by real-time ultrasound. All mated gilts were moved to individual gestation stalls located within the shed. Mated gilts were fed 2.2 kg of their respective treatment diet once a day at 0700 h until slaughter. A subset of 52 gilts were bled at 3 d postmating and at 25 d of pregnancy.

At 25.2 ± 0.1 d after mating, gilts were weighed, P2 backfat recorded and tattooed with an individual identification number. Gilts were slaughtered the following day at a commercial abattoir (Rivalea Australia, Corowa, NSW). Following exsanguination, the complete reproductive tract was recovered following evisceration for each animal. Only intact tracts were included in the data analysis for ovulation rate and embryo survival. The tracts were bagged, sealed and transferred to an adjacent laboratory where the number of corpora lutea (ovulation rate) and embryos were recorded per tract. Corpora albicans were not included in the measurement of ovulation rate. Embryos were counted and not classified further. Due to a weight limitation in the abattoir, a number of mated gilts (3 x Control; 3 x Omega-3 3 g/kg; 8 x Omega-3 10 g/kg) were unable to be slaughtered. In addition, one Omega-3 3 g/kg was erroneously sent to slaughter the day after mating. These were recorded as

mated in oestrus however there was no record of pregnancy, ovulation rate, embryo number or embryo survival.

5.2.3 Fatty acid analysis

Diets were sampled and 200 g per treatment diet and 5 mL plasma samples were stored at -20^{o}C for dietary fatty acid analysis at the Nutrition and Functional Foods Laboratory, The University of Adelaide, Waite Campus, SA. A subset of 18 pregnant gilts (6 per treatment) which had a complete set of blood samples from 24 weeks of age before treatment feeding commenced, 3 d and 25 d postmating were analysed by repeated measures for plasma fatty acids. Samples were analysed for fatty acids at the Nutrition and Functional Foods Laboratory, The University of Adelaide, Waite Campus, SA. A full description of the feed and plasma fatty acid analytical technique is provided in Chapter 2.

5.2.4 Statistical analysis

Data was analysed using SPSS v. 18.0 by General Linear Model Univariate ANOVA for live weight, backfat P2, age at mating, ovulation rate, embryo number and embryo survival. These continuous data parameters were distributed normally within treatment. The reproductive tract from one gilt allocated to Omega-3 10 g/kg was incompletely recovered at slaughter and was described as pregnant due to the presence of embryos in the remaining uterine horn, however the ovulation, embryo number and embryo survival values were excluded from the analysis. The proportion of the gilt numbers allocated to treatment at 24 weeks of age that were mated within 35 d of entry to the boar shed and the proportion of mated gilts recorded as pregnant at slaughter were analysed by chi-square. Fatty acid samples were analysed by GLM Univariate Model ANOVA using repeated measures. Where it was a significant contributor to the GLM model, block replicate (1-8) was included as a random factor. Pairwise tests for significance were performed using Least Significance

Difference test. Mean values are expressed as mean ± standard error (SE). Probability values $P < 0.05$ were described as significant, and $P < 0.10$ were used to describe trends.

5.3 Results

5.3.1 Plasma fatty acid response to supplementation

Before the start of dietary treatment feeding at 24 weeks of age, all gilts had similar plasma levels of ARA ($P = 0.582$), ALA ($P = 0.993$) and EPA ($P = 0.919$) (Figure 5.1). Sampled gilts allocated to Omega-3 3 g/kg commenced the dietary treatment with a higher level of LIN ($P < 0.05$) and a lower level of DHA compared to the Controls ($P < 0.05$). Gilts on Control and Omega-3 10 g/kg diets had plasma levels that were statistically similar in all fatty acids at the start of the experiment. By 3 d of pregnancy, the level of plasma EPA and DHA was substantially higher in gilts fed supplemented omega-3 diets at 3 and 10 g/kg (Figure 5.1). Plasma concentrations of EPA and DHA increased significantly with time ($P < 0.05$) in the supplemented treatments, but there was no difference in EPA and DHA levels in the Control group between the sampling points. Where treatment differences were not significant at $P < 0.05$ between gilts sampled at 25 d of pregnancy, there were trends ($P < 0.10$) for higher EPA and DHA levels in Omega-3 3 g/kg gilts compared to Controls (Figure 5.1). Plasma EPA levels in the gilts supplemented at 10 g/kg were 2.5-fold the level of the gilts supplemented at 3 g/kg and 5-fold the level of Control gilts by 25 d of pregnancy ($P < 0.001$). Similarly at 25 d, plasma DHA levels in the Omega-3 10 g/kg gilts were 1.85-fold and 3-fold the level recorded in Omega-3 3 g/kg and Control gilts, respectively ($P < 0.001$).

Over the sampling period from 24 weeks of age through to 25 d pregnancy, the concentration of ARA, LIN, EPA and DHA remained unchanged in the Controls (Figure 5.1). Gilts supplemented at 3 g/kg recorded a lower ARA plasma concentration by 25 d of pregnancy compared to the Controls (*P*

< 0.05). The Omega-3 10 g/kg gilts had a significantly lower ARA level at 3 d of pregnancy (P < 0.05). The concentration of plasma ARA also tended to be lower in the Omega-3 10 g/kg gilts compared to the Controls at 25 d of pregnancy (P = 0.055) . There was a significant decrease in the level of ALA in the Controls over time (P < 0.05) whereas ALA was unchanged in the gilts supplemented at either 3 or 10 g fish oil/kg of diet over time.

5.3.2 Treatment effect on live weight and fatness

All gilts were of similar weight and backfat P2 at treatment allocation and averaged 104.5 ± 0.5 kg with 10.3 ± 0.1 mm P2 at 24 weeks of age. There was an overall trend (P = 0.056) for omega-3 supplementation to affect mating weight (Table 5.3) with gilts at 10 g/kg being heavier at mating than those supplemented at 3 g/kg (P = 0.023), or Control gilts (P = 0.088). Mating weight of Omega-3 3 g/kg was similar to Control gilts. Daily weight gain during the *ad libitum* feeding period from 24 weeks of age to mating was not significantly affected by dietary treatment (0.857 ± 0.019 vs 0.836 ± 0.020 vs 0.863 ± 0.018 kg/day for Control, Omega-3 3 g/kg and Omega-3 10 g/kg respectively; P = 0.573). Weight at 25 d gestation was the same between Controls and Omega-3 3 g/kg, whereas Omega-3 10 g/kg gilts were heavier (P = 0.029, Table 5.3). There was no effect of dietary treatment on backfat P2 by mating or 25 d gestation.

5.3.3 Age at mating and onset of oestrous

The proportion of gilts that were mated on oestrus detected after 29 weeks of age and the proportion culled as anoestrous is summarised in Table 5.4. The proportion of gilts that commenced the study at 24 weeks of age and were mated by 36 weeks of age was low, however there was no difference between treatments. Dietary treatment tended to affect the age at mating in gilts (Table 5.4), such that pairwise comparisons between gilts on Omega-3 10 g/ kg were older at mating (225.0 ± 1.1 d) than Control gilts (221.5 ± 1.2 d of age, P = 0.035) and tended to be older than Omega-3 3 g/kg gilts (221.8 ± 1.3 d of age, P = 0.055).

The proportion of animals removed from the study was attributed to reproductive and physical causes. From all treatments, most of the gilt wastage was due to anoestrus (Table 5.4). There were no treatment effects on wastage or cause of wastage.

5.3.4 Ovulation rate and embryo survival

Reproductive performance from mated gilts assessed at 26.6 ± 0.3 d after mating is summarised in Table 5.5. There was no significant difference between dietary treatments on pregnancy outcome, ovulation rate, embryo number or embryo survival. Overall the pregnancy rates were high. There was a tendency for supplemented gilts to have a higher embryo survival (P = 0.070; Table 5.5) with an optimal response recorded in gilts on Omega-3 3 g/kg. The pairwise comparison of embryo survival between Control and Omega-3 3 g/kg was significant (81.8 ± 2.0% vs 88.9 ± 2.3%, respectively; P = 0.022). There was no difference in embryo survival between gilts on Omega-3 3 g/kg and Omega-3 10 g/kg (88.9 ± 2.3% vs 84.3 ± 2.1%, respectively; P = 0.388).

Table 5.1 Ingredient and calculated nutritional composition of experimental diets (g/kg as fed basis) fed to gilts allocated to either a control diet (Control), or an omega-3 PUFA diet containing 3 g fish oil/kg of diet (Omega-3 3 g/kg) or 10 g fish oil/kg (Omega-3 10 g/kg) until slaughter at 25.2 ± 0.1 d of pregnancy[1]

Ingredient, g/kg	Control	Omega-3	
	0 g/kg	3 g/kg	10 g/kg
Wheat	714	714	714
Millrun (wheat middlings)	174	174	174
Meat meal	52	52	52
Tallow[2]	30	27	20
Salt	2.0	2.0	2.0
Limestone	10.1	10.1	10.1
Water	10.0	10.0	10.0
Fish oil[3]	-	3.0	10.0
Synthetic lysine (L-lysine HCl)	3.8	3.8	3.8
Synthetic methionine (DL-methionine)	0.5	0.5	0.5
Synthetic threonine	1.4	1.4	1.4
Mineral vitamin premix[4]	2.4	2.4	2.4
Phytase	0.1	0.1	0.1
Xylanase	0.2	0.2	0.2
Monensin	1.0	1.0	1.0
Calculated nutrient analyses, g/kg			
Digestible energy (MJ/kg)	14.0	14.0	14.0
Crude protein	154.6	154.6	154.7
Crude fat[5]	56.6	56.6	56.6
Crude fibre	34	34	34
Calcium	8.1	8.1	8.1
Total phosphorus	5.4	5.4	5.4
Available phosphorus	4.0	4.0	4.0
Lysine	8.2	8.2	8.2
Ileal digestible lysine (g/MJ DE)	0.52	0.52	0.52
Methionine	2.7	2.7	2.7
Methionine + cystine	5.6	5.6	5.6
Threonine	5.9	5.9	5.9
Valine	6.4	6.4	6.4
Tryptophan	1.8	1.8	1.8

[1]Treatment diets commenced at 24 weeks of age of age and were fed *ad libitum* until mating. Diets were then fed at 2.2 kg/d until slaughter at 25.2 ± 0.1 d of pregnancy.

[2]Porcine derived tallow supplied by Biodiesel Producers, Barnawartha, VIC.

[3]Fish oil supplied by Feedworks, Sunbury, VIC.

[4]Premix provided the following nutrients (per kg air-dry diet): copper, 20 mg; iron, 80 mg; organic iron, 75 mg; manganese, 40 mg; zinc, 100 mg; iodine, 1 mg; selenium inorganic, 0.15 mg; selenium organic, 0.15 mg; chromium picolinate, 3.2 mg; betaine, 100 mg; antioxidant (Endox), 100 mg; vitamin A (retinol), 15,000 I.U.; vitamin D (cholecalciferol), 1,500 I.U.; vitamin E (α-tocopherol), 120 mg; vitamin B_2 (riboflavin), 3.5 mg; vitamin B_6 (pyridoxine), 2 mg; vitamin B_{12} (cyanocobalamin), 0.02 mg; biotin, 0.2 mg; folic acid, 0.5 mg; niacin, 20 mg; pantothenic acid, 5 mg.

[5]Analyzed crude fat levels: 53.8 g/kg (Control), 57.8 g/kg (Omega-3 3 g/kg) and 60.6 g/kg (Omega-3 10 g/kg).

Table 5.2 Fatty acid composition of treatment diets offered to gilts on Control, Omega-3 3 g/kg or Omega-3 10 g/kg from 24 weeks of age to early gestation (g/100 g total fatty acids as fed basis)[1]

Fatty acid (Common name)	Control	Omega-3	
	0 g/kg	3 g/kg	10 g/kg
C16:0 (palmitic acid)	20.9	20.4	19.1
C16:1 (palmitoleic acid)	1.97	2.10	2.56
C18:0 (stearic acid)	11.5	10.7	8.9
C18:1 (oleic acid)	28.4	27.7	27.0
C18:2 n-6 (α-linoleic acid)	29.5	29.9	29.0
C18:3 n-6 (γ-linolenic acid)	0.03	0.05	0.04
C18:3 n-3 (α-linolenic)	1.86	1.84	1.96
C20:4 n-6 (arachidonic acid)	0.16	0.17	0.21
C20:5 n-3 (eicosapentaenoic acid)	0.05	0.22	0.97
C22:5 n-3 (docosapentaenoic acid)	0.08	0.17	0.50
C22:6 n-3 (docosahexaenoic acid)	0.04	0.29	1.31
Total n-6	30.0	30.4	29.7
Total n-3	2.39	2.87	5.08
n-6:n3 ratio	12.6	10.6	5.8
Total saturated	36.1	34.7	31.8
Total transaturated	0.37	0.35	0.37

[1]Treatment diets commenced at 24 weeks of age of age and were fed *ad libitum* until mating. Diets were then fed at 2.2 kg/d until 25.2 ± 0.1 d of gestation.

Table 5.3 Mean ± SE live weight and backfat P2 of gilts fed unsupplemented diets (Control) or diets with omega-3 PUFA from fish oil at either 3 g fish oil/kg of diet or 10 g fish oil/kg of diet from 24 weeks of age through to 25 days of gestation[1]

	Control	Omega-3		
Item[2]	0 g/kg	3 g/kg	10 g/kg	*P* value
Number gilts	114	120	122	
Live weight, kg				
24 weeks age	104.5 ± 0.9	104.2 ± 0.9	105.0 ± 0.8	0.810
Mating[3]	150.0 ± 1.6	148.6 ± 1.7	153.9 ± 1.5	0.056
25 d gestation	159.1 ± 1.6[a]	159.1 ± 1.6[a]	163.9 ± 1.5[b]	0.043
Backfat P2, mm				
24 weeks	10.4 ± 0.2	10.3 ± 0.2	10.2 ± 0.2	0.829
Mating[3]	14.3 ± 0.4	14.3 ± 0.4	15.1 ± 0.4	0.206
25 d gestation	16.5 ± 0.4	16.2 ± 0.4	16.3 ± 0.4	0.841

[1]Treatment diets commenced at 24 weeks of age of age and were fed *ad libitum* until mating. Diets were then fed at 2.2 kg/d until 25.2 ± 0.1 d of gestation.
[2]Includes block as a random factor in GLM ANOVA model.
[3]Number recorded at mating were 76, 74 and 78 for Control, Omega-3 3 g/kg and Omega-3 10 g/kg, respectively.
[ab]Estimated marginal mean values within row with different superscripts are significant ($P < 0.05$)

Table 5.4 The proportion of gilts mated and the mating age, and the proportion of gilts removed from the herd as when gilts were fed unsupplemented diets (Control) or diets with omega-3 PUFA from fish oil at either 3 g fish oil/kg of diet or 10 g fish oil/kg of diet from 24 weeks of age through to 25 days of gestation[1]

	Control	Omega-3		
Item	0 g/kg	3 g/kg	10 g/kg	*P* value
Allocated gilts, no.	114	120	122	
Number gilts mated	76	74	78	
of allocated, %	66.7	61.7	63.9	χ^2 0.64, 0.728
Mating age, d	221.5 ± 1.2	221.8 ± 1.3	225.0 ± 1.1	0.061
Total number gilts removed,	38	46	44	
Of allocated, %	33.3	38.3	36.1	χ^2 0.64, 0.728
Number gilts anoestrous	32	39	39	
Of allocated, %	28.1	32.5	32.0	χ^2 0.64, 0.727

[1]Treatment diets commenced at 24 weeks of age of age and were fed *ad libitum* until mating. Diets were then fed at 2.2 kg/d until 25.2 ± 0.1 d of gestation.

Table 5.5 Pregnancy rates and mean ± SE ovulation rate and embryo number and embryo survival of gilts fed unsupplemented diets (Control) or diets with omega-3 PUFA from fish oil at either 3 g fish oil/kg of diet or 10 g fish oil/kg of diet assessed at slaughter at 26.2 ± 0.1 d of gestation[1]

	Control	Omega-3		
Item	0 g/kg	3 g/kg	10 g/kg	*P* value
Number gilts mated[2]	73	70	70	
Number gilts pregnant,	67	59	64	
Pregnancy rate, % mated	88.2	84.3	91.4	χ^2 2.62, 0.270
Ovulation rate, CL's[3]	16.9 ± 0.3	15.9 ± 0.4	16.8 ± 0.3[4]	0.113
Total embryos[3]	13.7 ± 0.4	14.1 ± 0.5	14.1 ± 0.4[4]	0.775
Embryo survival, %[3]	81.8 ± 2.0	88.9 ± 2.3	84.3 ± 2.1[4]	0.070

[1]Treatment diets commenced at 24 weeks of age of age and were fed *ad libitum* until mating. Diets were then fed at 2.2 kg/d for 25.2 ± 0.1 d of gestation.
[2]Numbers exclude mated gilts not assessed at slaughter.
[3]Allocation block included in GLM Model ANOVA as a random factor.
[4]Number of complete tracts analyzed was reduced to 61 in Omega-3 10 g/kg with three incomplete tracts excluded from analysis for ovulation rate, embryo number and embryo survival.

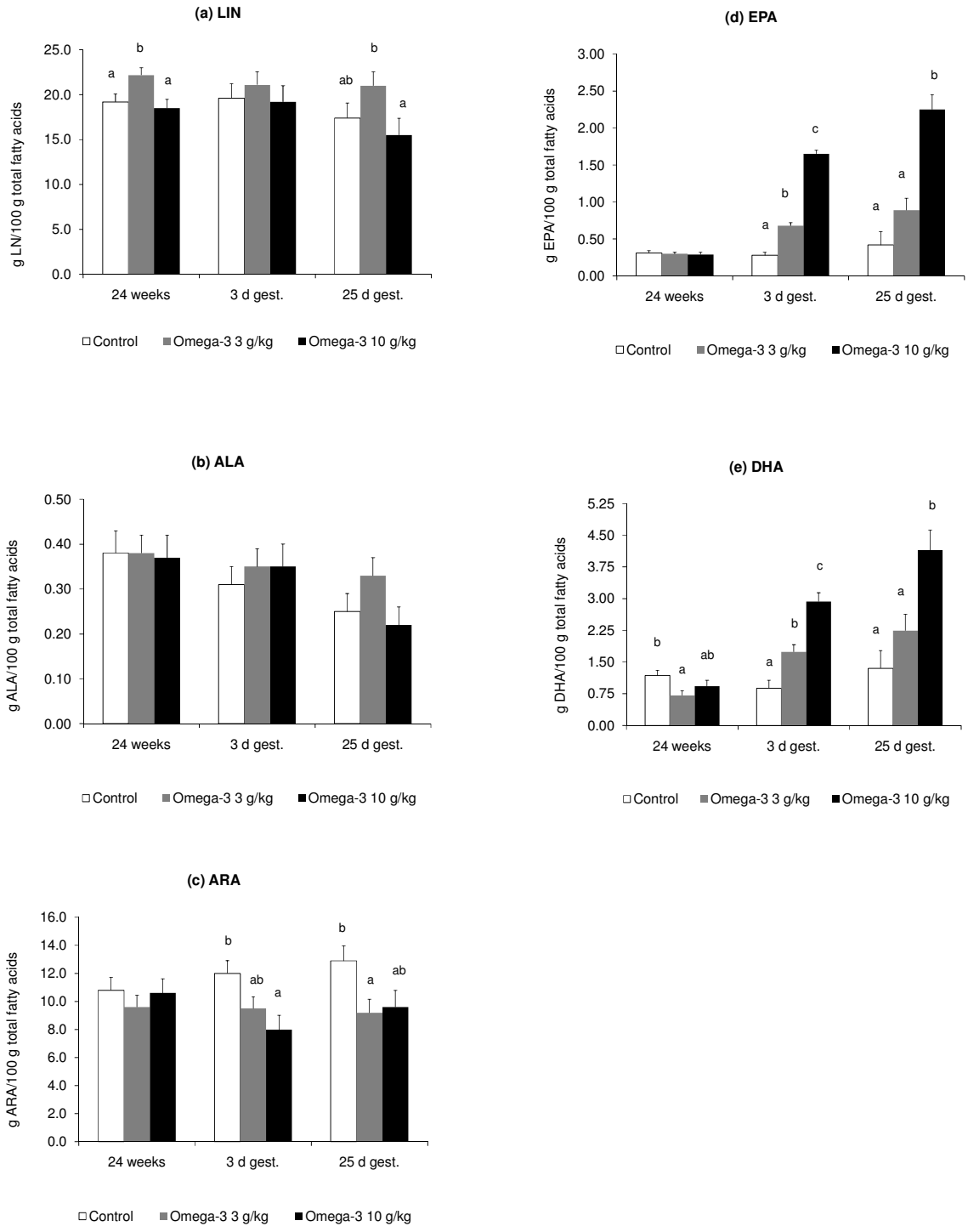

Figure 5.1 The fatty acid profile of gilts (n=6 per treatment) at 24 weeks of age (Day 0 of feeding) fed diets unsupplemented in omega-3 PUFA (Control) or increasing in dietary fish oil at 3 g fish oil/kg of diet or 10 g fish oil/kg diet on plasma fatty acids collected at 3 d and 25 d of gestation

[abc]Mean values within day of bleed sampling differ significantly between treatments ($P < 0.05$)

5.4 Discussion

The aim of the present study was to determine if embryo survival is improved when gilts were fed diets with a low or high level of omega-3 PUFA from fish oil from puberty and continued through early pregnancy. The results of this study showed whilst plasma levels of EPA and DHA were increased by dietary addition of fish oil, there was no effect of dietary omega-3 on the onset of oestrous, pregnancy, ovulation rate or embryo number in gilts. Furthermore, there was no evidence of a dose response to omega-3 supplementation on these reproductive parameters.

The addition of fish oil increased the plasma concentration of the long-chain omega-3 PUFA, EPA and DHA but decreased the level of the omega-6 PUFA, ARA. These results confirm findings in Chapter 4 that fish oil supplementation increases the plasma levels of long-chain omega-3 fatty acids but can also alter the balance of ARA, the favoured precursor of the eicosanoid pathway (James et al., 2000; Funk, 2001), especially at high levels (Smits et al., 2007).

Although there was change in the plasma PUFA balance and ARA by mating and 25 d of gestation, reproductive performance was largely unaffected by dietary treatment. Embryo survival tended to increase with supplementation, with the response optimized at 3 g fish oil/kg. Perez-Rigau et al. (1995) also reported an increase in embryo survival in gilts fed a diet supplemented with 4% menhaden fish oil fed before mating and continued in early gestation. In Chapter 4, litter size was not affected by supplementation of 3 g fish oil/kg fed to gilts during puberty and up to mating. Gilts were then fed an unsupplemented diet for the remainder of gestation. In the present study, the increase in embryo survival in Omega-3 3 g/kg gilts compared to Controls may be due to continuing the supplemented diet after mating. An increase in embryo survival may result in an increase in litter size born when gilts are fed continuously from puberty through to early pregnancy, although this is yet to be determined. However, given the relatively small increase in embryo survival and embryo

number at 23 d in the present study, the increase in parity 1 litter size may be small and insignificant. In both gilt studies in Chapter 4 and the present chapter, there was a positive effect of omega-3 supplementation on litter size. Reproductive performance in the gilts used in these studies were high and as such, may have precluded further improvements in reproductive performance due to omega-3 PUFA. In contrast, the baseline fertility was low in multiparous sows in Chapter 3 where subsequent litter size was improved with omega-3 supplementation. Therefore, the underlying level of fertility or specifically, embryo survival, may determine the size of the reproductive response to omega-3 PUFA.

Ovulation rate was unaffected by omega-3 supplementation. In their abstract, Webel et al. (2004) also reported that an increased litter size born in the parity subsequent to omega-3 supplementation during the previous gestation and lactation was due to an improvement in embryo survival, not ovulation rate. Embryo survival and maternal recognition of pregnancy requires adequate luteal support through high levels of progesterone around the time of implantation and early pregnancy (Geisert and Yelich, 1997; Ziecik et al., 2006). An increase in progesterone has been shown in some experiments in dairy cows when fed diets high in omega-3 PUFA from fish meal (Burke et al., 1997), however there are no reported studies on the effect of omega-3 PUFA on progesterone production. The reason for increased progesterone synthesis could be due to enhanced activity of the corpus luteum, possibly resulting from a greater cellular mass of the pre-ovulatory follicle. Larger corpora lutea were observed in cattle supplemented with omega-3 PUFA from linseed and linseed – fish oil supplements (Petit et al., 2002).

Estienne et al. (2006) proposed that there may have been insufficient supplementation of EPA and DHA in their study in gilts in which embryo survival was unaffected by omega-3 supplementation. These authors reported the supplemented diet provided EPA and DHA levels of 0.6 g/100 g total fatty acids, higher than the level of these PUFA in the omega-3 3 g/kg diet used in the present study.

The Omega-3 10 g/kg diet contained higher levels of EPA and DHA used by Estienne et al. (2006). However, the addition of 10 g fish oil to the diet in the present study did not increase reproductive performance in comparison to gilts fed the 3 g fish oil/kg or an unsupplemented diet. These findings confirm that dietary supplementation with 3 g fish oil/kg diet was sufficient to significantly increase plasma EPA, DHA and ARA in gilts and optimize embryo survival.

In a similar result to that reported in Chapter 4, a small delay in mating age was observed in the present study with dietary addition of fish oil, but only when supplemented at 10 g fish oil/kg. There was no further evidence of impaired ovarian function in gilts supplemented at 10 g/kg fish oil that might be expected with a delayed puberty, such as an increased incidence of anoestrous or a lower ovulation rate. There was no delay in the onset of puberty in gilts fed diets from 24 weeks of age containing 3 g fish oil/kg of diet which is in contrast to the findings in Chapter 4, where we reported that gilts fed diets containing 3 g fish oil/kg from 24 weeks of age were older at mating compared to controls. Together, the findings from Chapter 4 and the present study indicate that the effect of omega-3 supplementation on mating age is inconsistent.

In conclusion, the results of this experiment combined with those from Chapter 4 suggest the higher inherent fertility of gilts compared with sows in the study population used in both experiments may preclude further improvements in embryo survival and litter size due to omega-3 supplementation. Furthermore, supplementation at 3 g fish oil/kg optimizes embryo survival and concomitant litter size without the need to use higher levels of fish oil. These findings, together with the outcomes from Chapter 3 in sows, suggest that reproductive responses to omega-3 PUFA may be greater in older multiparous sows which have a lower level of embryo survival. Further experiments in this thesis therefore focused on defining the appropriate feeding duration for omega-3 supplementation to maximize the response in litter size, as well as examining the possible mechanisms governing this response.

Chapter 6 Effect of omega-3 supplementation on lactation and embryo survival in high parity sows

6.1 Introduction

In Chapter 3, it was reported that litter size increased when sows were fed omega-3 diets supplemented with low levels of fish oil 8 d prefarrowing and continued during lactation for 19 d. The result was similar to that reported by Palmer et al. (1970) who fed sows whole fish meal continuously throughout gestation, lactation and postweaning through to the subsequent gestation and second parity. Together these studies suggest that feeding sows a diet supplemented with omega-3 PUFA as fish oil for a relatively short period from prefarrowing through to weaning is sufficient to increase litter size. The results of Chapter 5 suggest that this increase in litter size is due to an improvement in embryo survival, possibly through an effect on oocyte developmental competence. However this remains to be determined.

In Chapter 5 there was a trend for increased embryo survival in gilts fed omega-3 supplemented diets. However the inherent level of fertility in the population was high and it was suggested that a larger response to supplementation may be observed in older sows having a low level of fertility. In Chapter 3, supplementation with omega-3 PUFA produced a large increase in subsequent litter size born (+10.3%) when multiparous sows were evaluated. The low litter size of mid parity and higher sows (> parity 5) is becoming a major limitation to lifetime sow performance (Hughes and Varley, 2003), reducing herd productivity and profitability (Dhuyvetter, 2000; Levis, 2005). As older sows are more likely to have a lower embryo survival than gilts (Foxcroft et al., 2006), it is proposed that sow litter size could be increased when sows are fed diets supplemented with omega-3 PUFA due to improved embryo survival. This would therefore increase sow longevity and lifetime performance.

In Chapter 3 supplementation ended at weaning, however extending the supplementation after weaning to mating could increase embryo survival through improved oocyte development and follicle growth prior to ovulation. In the previous chapter in gilts, there was evidence of an embryo survival response due to omega-3 supplementation. The aim of the present study therefore was to determine whether embryo survival and conception rate after implantation is improved with dietary supplementation of long-chain omega-3 PUFA from fish oil during lactation and postweaning in sows older than parity 5.

6.2 Materials and Methods

6.2.1 Animals and diets

The experiment was conducted at the Research and Innovation Unit, Rivalea Corowa, during winter and spring. Over eight weeks, 247 Large White x Landrace F1 sows were allocated to the experiment with an average parity 5.8 ± 0.1 (mean ± SE), ranging in age between parity 4 – 7 prior to farrowing. One hundred and twenty two sows were allocated to the Control treatment and 125 were allocated to Omega-3 treatment. Sows were randomized to treatment based on historical litter size, parity and farm source. In their 15th week of gestation (108.9 ± 0.2 d), pregnant sows were allocated to either an unsupplemented diet containing tallow (Control) or a diet where tallow was partially replaced with 3 g fish oil/kg of diet (Omega-3). Both diets were formulated using wheat and cereals and provided 14.9 MJ DE/kg; 186 g crude protein/kg; 0.50 g available lysine/MJ DE (Table 6.1). Diets were fed before farrowing, during lactation and continued after weaning up to mating. Thereafter all sows were fed a standard diet with no added fish oil or other omega-3 sources during pregnancy. Diet manufacture and ingredient suppliers of tallow and fish oil are detailed in Chapter 2. The level of supplementation was based on a recommended daily intake of 2.9 g of EPA and DHA (Leskanich and Noble, 1999). Diets were sampled and 200 g per treatment diet were stored at -

20°C for dietary fatty acid analysis at the Nutrition and Functional Foods Laboratory, The University of Adelaide, Waite Campus, SA (Table 6.2). A full description of the dietary fatty acid analytical technique is provided in Chapter 2.

6.2.2 Feeding and animal management

Sows were sourced from the commercial herd and were randomly housed within the farrowing rooms. On entry to the shed, 122 sows were allocated to the Control treatment and 125 sows were allocated to the Omega-3 treatment. Sows were fed 3 kg of their treatment diet once a day at 0700 h prior to and on the day of farrowing. Within 14 h of farrowing, litter size, number born live and dead (still born plus mummified fetuses) were recorded, and the total litter weighed (born live only). Fostering of piglets was practiced within dietary treatment and within 24 h after farrowing. Litter size was equalized within treatment averaging 10.1 ± 0.1 piglets. Litters were weighed after fostering was completed and again at 14 d of age and the day before weaning at 25.6 ± 0.2 d of age. Sows were weighed postfarrowing and at weaning and backfat P2 thickness measured by real-time ultrasound 65 mm from the midline over the last rib. Any sow that had her entire litter replaced due to illthrift (*e. coli,* poor weight gain) or removed due to mortality or agalactia was recorded and excluded from the data analysis during lactation, postweaning and subsequent litter performance. There was no creep feed provided to the piglets during lactation and each creep area was heated with a 175 W heat lamp.

From the day after farrowing, sows were offered their treatment diet up to four times a day to appetite. Feed delivery was adjusted to the sows' appetite on a daily basis during lactation commencing from d 1. Sows were weaned of their litters once a week at an average of 26.5 ± 0.2 d after farrowing. All sows were relocated to individual gestation housing and continued to be fed their treatment diet postweaning once a day at 3.0 kg until mating. On the third day after weaning, a mature boar was walked along the front of the sows each morning and when the sow exhibited a

standing heat response, the date of oestrus was recorded. Sows that did not display oestrus within 30 d of weaning were defined as anoestrous and culled. Sows in oestrus were artificially inseminated by two services 24 h apart (am/am). Following their first service all mated sows were fed 2.5 kg of the same gestation diet once a day (12.9 MJ DE/kg; 141 g crude protein/kg; 6.2 g lysine/kg; Table 6.1) until slaughter.

Mated sows were slaughtered at an average of 23.3 ± 0.1 d after mating and reproductive tracts were recovered following evisceration. Only intact tracts (both ovaries and intact uteri) were included in the data analysis for ovulation rate and embryo survival. Embryos were counted as those that were clearly visible (> 7 mm in length).

6.2.3 Statistical analysis

Data was processed through a general linear model analysis (GLM) of variance for treatments in a randomized design model assuming equal variance (Sokal and Rohlf, 1981). Data were analysed using SPSS v. 18.0 by General Linear Model Univariate ANOVA for live weight, backfat P2, ovulation rate, embryo number and embryo survival. Pairwise comparisons were analyzed using Least Significant Difference test. Block was included as a random factor in all GLM ANOVA models and lifetime total litter size born was included as an independent co-variate factor in the analysis of ovulation rate, embryo number and embryo survival. The proportion of sows that resumed oestrus and the proportion of sows mated that were confirmed pregnant during the examination of the reproductive tracts were analyzed by chi square. Mean values are expressed as mean ± standard error (SE). Probability values $P < 0.05$ were described as significant, and $P < 0.10$ were used to describe trends.

6.3 Results

6.3.1 Litter performance following prefarrowing dietary treatment

There was no parity difference between Control and Omega-3 sows (5.9 ± 0.1 vs 5.7 ± 0.1, respectively; *P* = 0.291). The period on dietary treatments prior to farrowing averaged 7.5 ± 0.2 d and there was no difference in gestation length observed between Control sows and Omega-3 sows (116.1 ± 0.2 d vs 116.3 ± 0.2 d; *P* = 0.420). The number of piglets born following the prefarrowing feeding period of 8 d is summarized in Table 6.3. Litter size born was similar between dietary treatments and there was no treatment effect on live born, still born or total born. Lactation performance was unaffected by dietary treatments with similar values in terms of piglet weight, weight gain and litter gain (Table 6.4).

6.3.2 Sow lactation feed intake and weight and backfat loss

Sow feed intake assessed over 26.6 ± 0.2 d of lactation was unaffected by dietary treatment (Table 6.5). Within the range included, parity had no significant effect on sow intake (*P* = 0.666) and there was no significant treatment x parity interaction (*P* = 0.180). Based on sow intake and dietary concentration of EPA and DHA, the calculated level of EPA and DHA intake was 2.75 g/d in Omega-3 sows. The Control sows had a calculated intake of 0.46 g/d EPA and DHA. After farrowing, sow live weight averaged 293.7 ± 1.8 kg and backfat P2 averaged 15.8 ± 0.3 mm across treatrments. By weaning at 26.6 ± 0.2 d, sows weighed 280.5 ± 1.7 kg with a backfat P2 thickness of 15.2 ± 0.2 mm. There was no difference between Control and Omega-3 sows in live weight or backfat P2 on d 1 or weaning (Table 6.5). Over lactation, there was no difference between Control and Omega-3 sows in sow weight loss or backfat P2 loss (Table 6.5) with sows losing 12.9 ± 1.1 kg and 0.4 ± 0.3 mm P2, respectively. Backfat loss across both treatments was highly variable.

6.3.3 Weaning to oestrus interval, pregnancy rate, ovulation and embryo survival

Sows from both treatments had a similar weaning to oestrus interval and there was no treatment effect on the proportion of sows resuming oestrous (Table 6.6). Sows on the Omega-3 treatment were fed their supplemented diet for an average of 39.0 ± 0.1 d. Mated sows were slaughtered at an average of 23.3 ± 0.1 d after the first insemination. One Control sow had an incomplete reproductive tract (missing one ovary) and was excluded from the records on ovulation rate, embryo number and survival. Pregnancy rate was low overall in the sow population studied, however there was no difference between treatments in the proportion of mated sows that were confirmed pregnant (Table 6.6). Omega-3 sows tended to have a significantly higher litter size and embryo survival than Control sows ($P < 0.06$) but there was no effect of treatment on ovulation rate (Table 6.6). The frequency distribution of litter size after 23 days was also observed to be influenced by diet (Figure 6.1), with a greater proportion of litters from Omega-3 sows having more than 17 embryos (χ^2 4.16; $P = 0.041$).

6.3.4 Sow removals

Of the 247 sows allocated to the study, 15 sows were removed from the study. For the Controls, there were two mortalities during lactation, three sows had poor litter performances and three were condemned at the abattoir (total of 8 sows). For the Omega-3 treatment there were two mortalities during lactation, two sows with poor litter performances, one sow taken off trial due to a mixed litter in the farrowing crate, and two abattoir condemnations (total of 7 sows). There was no effect of treatment on the number of sow removals or cause.

Table 6.1 Ingredient and calculated nutritional composition of experimental diets (g/kg as fed basis) fed to sows prefarrowing, during lactation and postweaning allocated to either a control diet (Control) or a diet containing omega-3 PUFA from 3 g fish oil/kg of diet (Omega-3)[1]

	Treatment diet		Commercial
Ingredient, g/kg	Control	Omega-3	Gestation
Wheat	565	565	502
Barley	93	93	210
Millrun (wheat middlings)	73.5	73.5	200
Canola meal	60	60	30
Soybean meal	40	40	-
Meat meal	56.7	56.7	-
Blood meal	10	10	-
Molasses	10	10	-
Tallow[2]	68	65	14.7
Salt	3.5	3.5	3.0
Limestone	9.0	9.0	15.0
Potassium chloride	4.17	4.17	-
Monensin	1.0	1.0	1.0
Fish oil[3]	-	3.0	-
Synthetic lysine (L-lysine HCl)	1.36	1.36	2.1
Synthetic threonine	0.15	0.15	1.7
Mineral vitamin premix[4]	2.3	2.3	2.3
Phytase	1.0	1.0	0.15
Betaine	1.0	1.0	-
Mycotoxin binder	1.0	1.0	2.0
Antioxidant	0.2	0.2	-
Calculated nutrient analyses, g/kg[2]			
Digestible energy (MJ/kg)	14.9	14.9	12.9
Crude protein	186	186	141
Crude fat	85	85	31
Crude fibre	36	36	45
Calcium	9.1	9.1	9.2
Total phosphorus	5.6	5.6	6.2
Available phosphorus	4.5	4.5	4.4
Lysine	9.0	9.0	6.2
Ileal digestible lysine (g/MJ DE)	0.50	0.50	0.40
Methionine	3.1	3.1	2.2
Methionine + cystine	6.3	6.3	5.1
Threonine	6.3	6.3	4.3
Valine	8.7	8.7	6.3
Isoleucine	6.1	6.1	4.5
Tryptophan	2.2	2.2	1.8

[1]Diets commenced 108.9 ± 0.2 d of gestation and fed 3 kg/d then continued *ad libitum* during lactation until weaning at 26.6 ± 0.2 d. Following weaning, sows continued to be fed their treatment diet 3 kg/d until mating. Thereafter all sows were fed 2.5 kg/d of the commercial gestation diet until slaughter at 23.3 ± 0.1 d of gestation.
[2]Tallow supplied by Biodiesel Producers, Barnawartha, VIC.
[3]Fish oil supplied by Feedworks, Sunbury, VIC.
[4]Premix provided the following nutrients (per kg diet as fed): copper, 20 mg; iron, 80 mg; organic iron, 50 mg; manganese, 40 mg; zinc, 100 mg; iodine, 1 mg; selenium inorganic, 0.15 mg; organic selenium, 0.15 mg; chromium picolinate, 3.2 mg; betaine, 2.5 g; antioxidant (Endox), 100 mg; vitamin A (retinol), 15,000 I.U.; vitamin D (cholecalciferol), 1,500 I.U.; vitamin E (α-tocopherol), 120 mg; vitamin B_2 (riboflavin), 3.5 mg; vitamin B_6 (pyridoxine), 2 mg; vitamin B_{12} (cyanocobalamin), 0.02 mg; biotin, 0.2 mg; folic acid, 0.5 mg; niacin, 65 mg; pantothenic acid, 5 mg.

Table 6.2 Fatty acid composition of treatment diets offered to sows during lactation (g/100 g total fatty acids as fed basis)[1]

Fatty acid (Common name)	Control	Omega-3
C16:0 (palmitic acid)	23.1	22.8
C16:1 (palmitoleic acid)	2.49	2.42
C18:0 (stearic acid)	16.1	16.1
C18:1 (oleic acid)	32.7	32.6
C18:2 n-6 (α-linoleic acid)	14.4	13.7
C18:3 n-6 (γ-linolenic acid)	0.03	0.03
C18:3 n-3 (α-linolenic)	1.60	1.59
C20:4 n-6 (arachidonic acid)	0.14	0.14
C20:5 n-3 (eicosapentaenoic acid)	0.03	0.20
C22:5 n-3 (docosapentaenoic acid)	0.11	0.17
C22:6 n-3 (docosahexaenoic acid)	0.04	0.22
Total n-6	14.7	14.1
Total n-3	1.90	2.30
n-6:n3 ratio	7.73	6.13
Total saturated	36.1	36.4
Total transaturated	2.95	3.02

[1]Diets commenced 108.9 ± 0.2 d of gestation and fed 3 kg/d then continued *ad libitum* during lactation until weaning at 26.6 ± 0.2 d. Following weaning, sows continued to be fed their treatment diet 3 kg/d until mating.

Table 6.3 Litter size of piglets born to sows fed for 7.5 ± 0.2 d prefarrowing either a Control diet or Omega-3 diet containing omega-3 PUFA from 3 g of fish oil/kg of diet

	Treatment[1]			
Item[2]	Control	Omega-3	SEM	*P* value
Number litters farrowed	122	125		
Born live, pigs/litter	10.1	10.6	0.2	0.279
Still born, pigs/litter	1.9	1.8	0.1	0.569
Total born, pigs/litter	12.0	12.4	0.2	0.518

[1]Diets commenced 108.9 ± 0.2 d of gestation and fed 3 kg/d then continued *ad libitum* during lactation until weaning at 26.6 ± 0.2 d. Following weaning, sows continued to be fed their treatment diet 3 kg/d until mating.
[2]Data recorded on birth sow within 24 h of farrowing.

Table 6.4 Average piglet and litter daily gain between d 1 and prior to weaning at 25.6 ± 0.2 d of age when sows were fed a Control diet or Omega-3 diet containing 3 g fish oil/kg of diet prefarrowing, during lactation and postweaning to mating

	Treatment[1]			
Item	Control	Omega-3	SEM	*P* value
Piglet d 1 live weight, kg	1.48	1.53	0.02	0.165
Piglet d 14 live weight, kg	4.40	4.46	0.05	0.669
Piglet weaned weight, kg[2]	7.14	7.16	0.08	0.759
Piglet ADG d1 to wean, kg/d[2]	0.222	0.220	0.003	0.839
Litter daily gain, kg/sow[2]	1.73	1.72	0.04	0.950

[1]Diets commenced 108.9 ± 0.2 d of gestation and fed 3 kg/d then continued *ad libitum* during lactation until weaning at 26.6 ± 0.2 d. Following weaning, sows continued to be fed their treatment diet 3 kg/d until mating.
[2]Estimated marginal mean value with age at weaning weigh period included as a covariate (25.5 ± 0.2 d of age).

Table 6.5 Sow lactation feed intake and live weight and back fat P2 between d 1 and weaning at 26.5 ± 0.2 d of age when sows were fed a Control diet or Omega-3 diet containing 3 g fish oil/kg of diet prefarrowing, during lactation and postweaning to mating

	Treatment[1]			
Item	Control	Omega-3	SEM	*P* value
Sow feed intake, kg/d	7.8	7.7	0.1	0.318
Sow d 1 live weight, kg	292.0	293.7	1.8	0.675
Sow wean live weight, kg	281.0	280.0	1.7	0.805
Sow d 1 to wean weight loss, kg	13.0	12.9	1.1	0.957
Sow d 1 backfat P2, mm	15.8	15.8	0.3	0.931
Sow wean backfat P2, mm	15.1	15.4	0.2	0.528
Sow d 1 to wean P2 loss, mm	0.5	0.3	0.3	0.743

[1]Diets commenced 108.9 ± 0.2 d of gestation and fed 3 kg/d then continued *ad libitum* during lactation until weaning at 26.6 ± 0.2 d. Following weaning, sows continued to be fed their treatment diet 3 kg/d until mating.

Table 6.6 Weaning to oestrus interval, pregnancy rate, ovulation and embryo recovery in sows fed Control and Omega-3 sows fed diets supplemented with 3 g fish oil/kg of diet prefarrowing, during lactation and postweaning to mating and slaughtered at 23.3 ± 0.1 d of gestation

	Treatment[1]			
Item	Control	Omega-3	SEM	*P* value
Number sows weaned	117	120		
Wean to oestrus, d	5.3	5.0	0.2	0.433
Number sows mated	93	92		
Oestrous resumption, % weaned	79.5	76.7		χ^2 0.28, 0.600
Number sows pregnant[2]	51	57		
Pregnancy rate, % mated[2]	56.7	63.3		χ^2 0.83, 0.361
Ovulation rate[3]	22.2	22.2	0.4	0.995
Embryos recovered[3]	13.6	15.5	0.4	0.055
Embryo survival, %[3]	61.5	70.0	1.8	0.054

[1]Diets commenced 108.9 ± 0.2 d of gestation and fed 3 kg/d then continued *ad libitum* during lactation until weaning at 26.6 ± 0.2 d. Following weaning, sows continued to be fed their treatment diet 3 kg/d until mating. All sows were fed the same commercial gestation diet 2.5 kg/d until slaughter 23.3 ± 0.1 d of gestation.
[2]Number of Control sows mated excludes 3 sows condemned. Number of Omega-3 sows excludes 2 sows condemned. Reproductive tracts were unrecovered from condemned sows.
[3]Estimated marginal mean values with lifetime total born included as a covariate factor (11.3 ± 0.1).

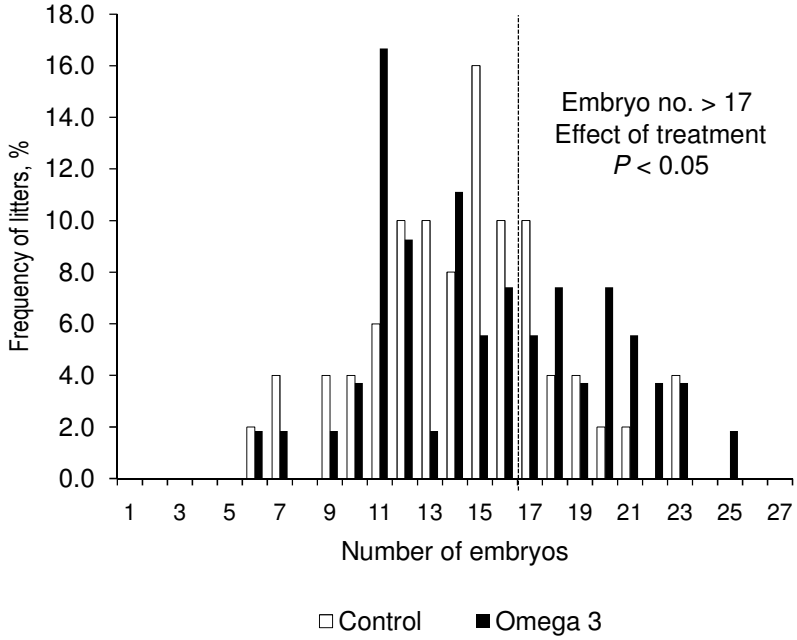

Figure 6.1 The frequency (%) of recovered embryos per sow following feeding prefarrowing, during lactation and postweaning to mating a diet containing no fish oil (Control) or an omega-3 diet containing 3 g fish oil/kg of diet (Omega-3) at 23.3 ± 0.1 d gestation

6.4 Discussion

The aim of this experiment was to determine if the increase in subsequent litter size observed in Chapter 3 in sows supplemented with omega-3 PUFA from fish oil in the preceding lactation was due to an increase in ovulation rate or embryo survival. The results of the present study showed that feeding sows a diet high in long-chain omega-3 PUFA for 39 days from prefarrowing until mating did not increase ovulation rate but did increase the number of foetuses present at 23 d of gestation. This suggests that the increase in subsequent litter size observed in supplemented sows in Chapter 3 was due to an increase in embryo survival as observed in the present study. The inclusion of 3 g fish oil/kg of diet oil as a partial replacement for tallow in the diet did not affect feed intake of sows or tissue loss over 27 d lactation, supporting our findings in Chapter 3 where lactation length was 19 d. This is the first study to our knowledge that an increase in embryo survival has been reported for sows fed isocaloric diets high in long-chain omega-3 PUFA from prefarrowing through to mating.

These findings are in contrast to the outcomes reported by Perez-Rigau et al. (1995) who fed parity 3 sows a diet containing menhaden fish oil or starch from weaning until slaughter at six weeks of gestation and reported no effect of diet on embryo survival or ovulation rate in sows (Perez-Rigau et al., 1995). In gilts we failed to detect a significant response to omega-3 PUFA on litter size and farrowing rate (Chapter 4), although when the effect of supplementation on embryo survival was investigated, there was a trend for a 9% improvement in embryo survival in gilts fed a diet with 3 g fish oil/kg of diet (Chapter 5). In the present study, we hypothesized that the size of the response to long-chain omega-3 PUFA from fish oil would be greater in older sows compared to gilts, as older sows have a lower embryo survival (Foxcroft et al., 2006). The findings from the present study support this, with an increase in embryo survival of 14% in the Omega-3 sows compared with Control sows. While the present study did not allow for embryo survival in gilts and sows to be compared, the embryo survival in the control sows (61.5%) in the present study was much lower

than for control gilts (81.8%) of the same genotype in Chapter 5. Estienne et al. (2006) suggested that supplementation with omega-3 PUFA may not have enhanced reproduction in their gilts because they had a high inherent fertility. In our gilt studies, total litter size born (Chapter 4) and embryo number (Chapter 5) was only increased by 3% in response to feeding fish oil. Together, these findings support the hypothesis that the response to omega-3 PUFA is more likely to be higher in older sows due to a lower level of embryo survival compared to gilts.

There are few published studies on the effect of the source of dietary energy on ovulation and embryo survival at constant levels of dietary energy intake. For example Kemp et al. (1995) reported there was no effect on ovulation rate or embryo survival when sows were fed a diet high in soybean oil compared to starch. Others that compared the response between dietary fat source as tallow or either starch (van den Brand et al., 2000) or corn oil (Park et al., 2008) reported no effect on weaning to oestrus interval, pre-ovulatory LH, progesterone, ovulation rate or embryo survival.

Where dietary responses have been recorded to changes in energy or fat source, it has been associated with changes in lactation intake or live weight loss over lactation (e.g. King and Williams, 1984). In the present study we did not observe any change in sow weight or condition during lactation. Consistent with our previous study in multiparous sows in Chapter 3, sow feed intake during lactation was unaffected by the supplementation level of 3 g fish oil/kg of diet. These findings support those of Mateo et al. (2009) who reported no change in lactation intake to a diet enriched with omega-3 PUFA from fish oil fed to gilts and first parity sows from 60 d of gestation through to weaning at 21 d of age. Together with our own findings in the present study and in Chapter 3, we conclude that the reproductive response observed due to increased dietary omega-3 PUFA occurs without a change in energy intake or mobilization of tissue reserves during lactation.

There was no significant effect on live born, still births or total born following short-term prefarrowing feeding of the supplemented diet, a result similar to Chapter 3. Others have fed sows with diets

containing fish oil from 60 d gestation up to farrowing and reported no difference in litter size born between supplemented and non-supplemented sows (Rooke et al., 2001b; Rooke et al., 2001c; Mateo et al., 2009). Consistent with our previous findings, there were no effects on lactation performance as measured by piglet weight gain or weaned weight. Litter size following the short period of dietary introduction prefarrowing was also unaffected. Although long-chain omega-3 PUFA are regarded by some as essential for piglet growth and immunity development (Leskanich and Noble, 1999), higher intakes may be required for a response in piglet performance (Rooke et al., 2001c). The duration of supplementation prior to farrowing may also be a factor affecting piglet growth in lactation. In first parity sows fed an omega-3 supplemented diet from 60 d gestation and continued through lactation to weaning, Mateo et al. (2009) reported an increase in piglet and litter weight gain in the omega-3 supplemented sows between d 10 to 21 d of lactation. These authors related increased litter growth during lactation to be a result of improved piglet development and an increase in the immunoglobulin levels of colostrum due to omega-3 supplementation during mid and late gestation. In the present study, introduction to the omega-3 diet commenced much later at 109 d of gestation, and may not have been enough time prior to farrowing to affect piglet brain development and suckling behavior, or colostrum composition.

The mechanism by which long-chain omega-3 PUFA improves embryo survival remains to be determined. Embryo survival is a function of oocyte quality, or developmental competence, and embryo growth and development to implantation (Zak et al., 1997b; Ashworth and Antipatis, 1999; Ferguson et al., 2006). While the present study has demonstrated no change in ovulation rate, there may still be effects of omega-3 PUFA on oocyte metabolism. In a parallel study to the present one, we recovered oocytes from unmated Control or Omega-3 sows fed diets prefarrowing and continued to 4 d postweaning and found that embryo development to blastocyst stage and cell number was greater in the omega-3 sows (Mitchell et al., 2010, abstract). Pig ooyctes and follicular fluid are known to contain high levels of PUFA (Yao et al., 1980; Homa et al., 1986; Khandoker et al., 1997),

hence supplemental dietary omega-3 PUFA may be required for optimal follicular growth and oocyte development as has been demonstrated in ruminants (Petit et al., 2002; Zeron et al., 2002). The increase in embryo survival in the present experiment may have been due to improved oocyte quality as a result of increased omega-3 PUFA before ovulation, as observed in-vitro by Mitchell et al. (2010). Alternatively, omega-3 PUFA prior to ovulation influences progesterone production from the subsequent corpus luteum, which in turn affects implantation rate, possibly due to altered prostaglandin synthesis (Waclawik et al., 2009). These possible mechanisms will be investigated in the following chapters.

In conclusion, the present study showed that dietary supplementation of long-chain omega-3 fatty acids from fish oil prefarrowing through to mating increased embryo survival and potential litter size in sows older than parity 5. This response was independent of energy intake and supports the previous finding in Chapter 3 where fish oil supplemented diets fed to sows from 8 d prefarrowing and during 19 d of lactation increased subsequent litter size born.

Chapter 7 The effect of omega-3 PUFA supplementation from fish oil fed during lactation and through to day 28 of gestation on subsequent reproductive performance in sows

7.1 Introduction

In Chapter 3 litter size was increased by a pig per litter live born and total born when sows were fed a lactation diet supplemented with long-chain omega-3 PUFA from fish oil prefarrowing and during lactation. In Chapter 6, this response was due to a 14% increase in embryo survival as ovulation rate was unaffected. The results of Chapter 4 and 5 demonstrated that supplementation had no effect on litter size in gilts which we concluded was due to their relatively high inherent fertility, suggesting that the response may be higher in older parity sows where embryo survival, rather than ovulation rate, is limiting (Foxcroft et al., 2006).

One of the causes contributing to high sow turnover in a breeding herd is low reproductive performance. Over the years there have been several studies that have concluded that reproductive failure and low fecundity contribute half of the sows culled in a herd (Hughes and Varley, 2003). Engblom et al. (2008) reported that culling for low litter size was accentuated as parity increased in commercial herds. Historically, the fertility of sows continued to increase with age until parity 4 or 5, before declining with a productive life expectancy averaging 8 or 9 litters (Tummaruk et al., 2001; Levis, 2005). However, more recent studies of farm records show that reproductive performance is now peaking at younger parities, before a rapid decline (Hughes and Varley, 2003; Rodriguez-Zas et al., 2003).

The inclusion of fish oil to supplement the diet with long-chain omega-3 PUFA may be a nutritional strategy for overcoming declining reproductive performance in older sows. The mode of action for

omega-3 PUFA in improving embryo survival remains unclear, however it may be a result of a combination of factors including an improved oocyte quality (Zeron et al., 2002; Mitchell et al., 2010) and/or changes to the uterine environment during implantation due to alterations in prostaglandin synthesis and progesterone production (Waclawik et al., 2009). In Chapter 5, embryo survival was increased in gilts fed an omega-3 supplemented diet with 3 g fish oil/kg in gilts before mating and during the first four weeks of pregnancy. The improvement in sow litter size observed in Chapter 6 when sows were fed supplemented diets during lactation and up to mating may be greater if omega-3 diets continue to be fed in early pregnancy when embryo development and implantation occurs.

The identification of the stage in the reproductive cycle where omega-3 PUFA has its effect has not only economic benefits in terms of cost-effective feeding strategies, but also provides further insight into the possible mechanisms involved. The aim of the present study therefore was to determine if litter size and farrowing rate is increased by omega-3 PUFA when fed for a period of supplementation either for four weeks prior to mating and/or the first four weeks of gestation in multiparous sows.

7.2 Materials and Methods

7.2.1 Animals and diets

The experiment was conducted on a commercial piggery during winter and spring. Over five weeks, 1,216 Large White x Landrace F1 sows ranging in age between parity 0 (pregnant gilts) and parity 6 prior to farrowing (2.7 ± 0.1, mean ± SE) commenced the experiment when they entered the farrowing shed at 112.1 ± 0.1 d gestation. Sows were allocated to either a lactation diet with no added fish oil (Control) or a lactation diet supplemented in omega-3 PUFA from the partial replacement of tallow with 3 g fish oil/kg diet (Omega-3). Lactation diets were fed prefarrowing from

entry to the farrowing shed, during lactation and postweaning to mating and were formulated using wheat, cereals, and soybean and canola meals and provided 14.9 MJ DE/kg; 188 g crude protein/kg; 0.51 g available lysine/MJ DE (Table 7.1).

After weaning, 860 mated sows from the study group (average parity 3.7 ± 0.1, ranging in parity from 1 – 7 at weaning) were fed either an unsupplemented gestation diet (Control) or an omega-3 diet containing 6 g fish oil/kg of diet (Omega-3) partially replacing tallow in a 2 x 2 factorial design with lactation and gestation treatment feeding regimens as follows: Control – Control; Omega-3 – Control; Control – Omega-3; Omega-3 – Omega-3 (Figure 7.1). Gestation diets were formulated using wheat and cereals and provided 12.9 MJ DE/kg; 144 g crude protein/kg; 0.38 g available lysine/MJ DE (Table 7.1). Other essential amino acids were formulated as a ratio to lysine in all treatment diets. Diets were sampled and 200 g per treatment diet were stored at -20^{o}C for dietary fatty acid analysis at the Nutrition and Functional Foods Laboratory, The University of Adelaide, Waite Campus, SA (Table 7.2). A full description of the dietary fatty acid analytical technique is provided in Chapter 2.

7.2.2 Feeding and animal management

There were 608 sows that commenced the study and fed an unsupplemented lactation diet (Control) and 608 sows fed the omega-3 supplemented diet for approximately 3 days prior to farrowing. Sows were fed 3 kg of their respective lactation diet once a day until d 2 of lactation (the day following farrowing). Sows were fed their treatment diet during lactation *ad libitum* from d 2 of lactation until weaning at 19.8 ± 0.1 d after farrowing. To minimize bias due to pen location within the shed, the treatment allocations on each half of the shed were alternated between weekly blocks. Data on previous litter size born live and total born (including mummified foetuses and stillborns), and parity were retrieved from farm records and current date of farrowing, litter size born and pen location were recorded. Sows were managed under commercial practice and within the first 24 h of farrowing, litter size was adjusted within treatment according to rearing ability and ranged in number from 8 to 14.

Some sows were weaned early due to milking failure, sudden death or destruction due to injury. In some cases, sows had an extended lactation if they were used as nursing sows to maintain the litter. In these cases, the nursing sow was shifted to a pen and continued on her respective treatment. Sows that had a shortened lactation (< 10 d) due to milking failure or poor mothering ability, and nurse sows that had an extended lactation (> 32 d) were excluded from the analysis of performance parameters subsequent to weaning.

Of the 1,136 sows weaned, 398 Control and 462 Omega-3 sows were housed in individual stalls in a designated study area of the piggery. Not all the sows commencing the experimental diets in lactation were able to be retained for postweaning treatment allocation due to space limitations within the facility. There were more Omega-3 sows available to continue the experiment due to the management of the weaning, which was unintentional. The characteristics of the subset in terms of litter size weaned, lactation length and parity were the same as the population weaned. At weaning each week, stalled sows on their respective treatment were blocked in the shed to assist in the feeding of treatment diets and continued to receive their lactation diet until mating. Sows were fed 2.5 kg once a day at 0700 h until mating.

Sows were mated at their first oestrus after weaning by artificial insemination in an am/am service, 24 h apart. Once mated, half the sows were allocated to a Control gestation diet and the other half were allocated to an Omega-3 gestation diet according to Figure 7.1. Sows remained in the study area and continued to be fed their gestation treatment diet from d 1 (24 h after first AI) until pregnancy was confirmed by ultrasound at approximately 28 d. All sows ended their experimental treatment diets at this point. After 28 d gestation, sows were moved to individual stalls outside of the study area and fed 2.7 kg/d a commercial gestation diet (Table 7.1) until entering the farrowing sheds for the birth of their subsequent litter.

Mated sows were checked for oestrus after mating using a mature boar commencing 18 d after mating. At approximately 28 d, sows were tested for pregnancy using real time ultrasound and re-tested the following week as confirmation. Reason for sow removal, subsequent farrowing rate (defined as the proportion of sows that farrowed following mating on their first postweaning oestrus), and subsequent litter size born were recorded within 24 h of birth. Total litter size is defined as the sum of live born, still born and mummified foetuses.

7.2.3 Statistical analysis

Data was processed through a General Linear Model (GLM) analysis of variance for treatments in a randomized design model assuming equal variance (Sokal and Rohlf, 1981). In the analysis of subsequent litter size, only the 860 sows that remained in the study area postweaning and comprised the 2 x 2 design were included. Any sow that returned to service and was re-mated was considered a failed pregnancy in the farrowing rate data analysis and their subsequent litter performance was excluded. The subsequent litter data was normally distributed and was analyzed by GLM Univariate ANOVA with replicate and parity included as random factors. Variables including lactation length and average lifetime litter size were included as covariates in the model where stated in the tables. Pairwise comparisons for each dependent variable between treatments were performed using Least Significant Difference test. The resumption of oestrous, farrowing rate and reasons for sow removal were analyzed by chi square. Probability values < 0.05 were described as significant and $P < 0.10$ were used to describe trends.

7.3 Results

7.3.1 Effects of lactation diets on sows weaned and postweaning onset of oestrus

From the production records, there was no evidence of any treatment effects on lactation performance or sow mortality. Of the 1,216 sows allocated to the study prefarrowing, 26 sows were taken off the study during lactation due to milking failure (15 x Control, 11 x Omega-3) and 42 sows for extended lactations due to being used as nurse sows (21 x Control, 21 x Omega-3). There were seven sows removed due to mortality during lactation (3 x Control, 4 x Omega-3) and five for management reasons including high still births and poor udders (4 x Control, 1 x Omega-3). The litter size weaned was unaffected by dietary treatment and averaged 9.0 ± 0.1. There was no difference in the proportion of sows weaned and subsequently mated between Control or Omega-3 treatments (Table 7.3). The interval from weaning to oestrus was similarly unaffected by dietary treatment (average 7.0 ± 0.2 d, P = 0.268). The main reasons for failure to mate were due to management decisions relating to physical issues (locomotion, damaged udder, body condition, infectious discharge: 13 x Control, 17 x Omega-3; χ^2 0.51, P = 0.477), or mortality (sudden death & euthanasia: 2 x Control, 4 x Omega-3; χ^2 0.65, P = 0.420). Out of 1,136 sows weaned only three failed to resume oestrus within six weeks postweaning (1 x Control, 2 x Omega-3, χ^2 0.32, P = 0.569).

7.3.2 Subsequent reproductive performance

Subsequent reproductive performance and litter size of the 860 sows that remained on the 2 x 2 factorial design after mating is summarized in Table 7.4. The subsequent farrowing rates were similar between treatments (P = 0.327). There were no differences in the causes of mating failure between treatments: return to oestrus = 4.8% (χ^2 0.42, P = 0.935); pregnancy tested negative = 5.2% (χ^2 3.27, P = 0.352); mortality = 4.0% (χ^2 4.94, P = 0.176); late pregnancy failure & abortion = 2.0% (χ^2 5.08, P = 0.166); infectious discharge = 1% (χ^2 1.30, P = 0.729).

Subsequent litter size total born was significantly different between treatments ($P < 0.05$; Table 7.4). Pairwise comparison testing confirmed that Control – Omega-3 sows and Omega-3 – Omega-3 sows had a higher litter size total born compared to the unsupplemented Controls – Control sows ($P < 0.05$). Subsequent total born for sows fed as Omega-3 – Control was intermediate. Subsequent litter size born live was not statistically affected by treatment but numerically followed a similar treatment response as total born (Table 7.4). Stillbirths tended to be higher ($P = 0.10$) in Omega-3 – Omega-3 sows and was associated with a higher total number of pigs born (Table 7.4).

There was no overall treatment x parity interaction in subsequent litter size total born ($P = 0.570$). Due to the small number of sows weaned within each parity x treatment cohort, the data for subsequent litter size born was further analyzed for each dietary treatment regimen in first-litter sows (parity 1), mid-parity sows (parity 2 and 3) and older sows (parity 4 – 7). There was a significant response in subsequent litter size total born in the older sow parities allocated to Omega-3 either before or after mating when the dataset was further separated into the parity categories defined above at the time of weaning (Table 7.5). The increase in subsequent total born was greatest in the sows fed supplemented diets in lactation through to four weeks gestation (Omega-3 – Omega-3). Subsequent total born in the Control – Omega-3 sows were similar to Omega-3 – Omega-3 sows and Omega-3 – Control, and significantly higher than sows on Control – Control (Table 7.5). Sow farrowing rates were similar between Control – Control, Control – Omega-3, Omega-3 – Control and Omega-3 – Omega-3 treatments in the older sow category (82.1%, 77.9%, 82.4%, 81.5% respectively; χ^2 0.83, 0.842). The subsequent total born of weaned parity 1 sows and mid-parity sows was unaffected when fed supplemented diets during either lactation, early pregnancy, or both (Table 7.5). Within each parity treatment comparison, subsequent live born was not significantly affected, but followed a similar response pattern as total born (Table 7.5). Subsequent sow farrowing rates for Control – Control, Control – Omega-3, Omega-3 – Control and Omega-3 –

Omega-3 were also similar for weaned parity 1 (91.3%, 88.9%, 88.0%, 84.2% respectively; χ^2 0.51, P = 0.916) and parity 2 – 3 sows (82.2%, 77.9%, 88.0%, 87.9% respectively; χ^2 4.60, P = 0.330).

Figure 7.2 illustrates the pattern for total number of piglets born subsequent to the treatments imposed as parity increased. In parity 4, 5 and 6, subsequent litter size total born was significantly higher in Omega-3 – Omega-3 sows compared to Control – Control and Omega-3 – Control sows (P < 0.05).

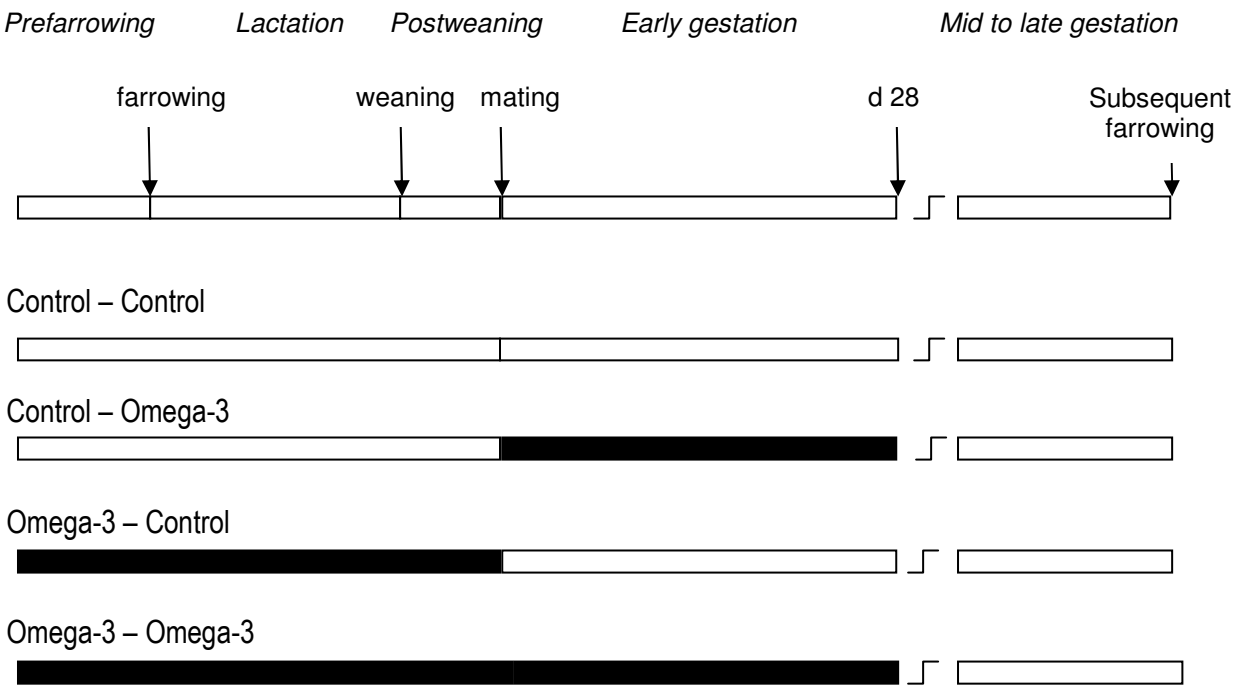

Figure 7.1 Schematic representation of treatment feeding regimen of unsupplemented lactation and gestation diets (Control□) and omega-3 supplemented diets as fish oil (Omega-3■)

Table 7.1 Ingredient and calculated nutritional composition of experimental lactation diets fed prefarrowing and during lactation and gestation diets fed after mating until 28 d (as fed basis)

	Lactation[1]		Gestation[1]		Commercial
Ingredient, g/kg	Control	Omega-3	Control	Omega-3	gestation
Wheat	563	563	496	496	496
Barley	93	93	246	246	246
Millrun (wheat middlings)	74	74	190	190	190
Canola meal	60	60	-	-	-
Soybean meal	40	40	-	-	-
Meat meal	57	57	17	17	17
Blood meal	10	10	-	-	-
Molasses	10	10	-	-	-
Tallow	68	65	10	4	10
Water[2]	-	-	10	10	10
Salt	3.5	3.5	3.0	3.0	3.0
Limestone	9.0	9.0	12.5	12.5	12.5
Dicalcium phosphorus	-	-	9.0	9.0	9.0
Potassium chloride	4.2	4.2	-	-	-
Monensin	1.0	1.0	1.0	1.0	1.0
Fish oil[3]	-	3.0	-	6.0	-
Synthetic lysine (L-lysine HCl)	1.36	1.36	1.53	1.53	1.53
Synthetic threonine	0.15	0.15	0.20	0.20	0.20
Mineral vitamin premix[4]	2.2	2.2	2.2	2.2	2.2
Phytase	1.0	1.0	1.0	1.0	1.0
Zinc bacitracin	2.5	2.5	-	-	-
Betaine	1.0	1.0	-	-	-
Mycotoxin binder	0.5	0.5	0.5	0.5	0.5
Antioxidant	0.2	0.2	-	-	-
Calculated nutrient analyses, g/kg					
Digestible energy (MJ/kg)	14.9	14.9	12.9	12.9	12.9
Crude protein	188	188	144	144	144
Crude fat[5]	85	85	27	27	27
Crude fibre	36	36	42	42	42
Calcium	9.1	9.1	9.3	9.3	9.3
Total phosphorus	5.6	5.6	6.5	6.5	6.5
Available phosphorus	4.5	4.5	4.9	4.9	4.9
Lysine	9.0	9.0	6.0	6.0	6.0
Ileal digestible lysine (g/MJ DE)	0.51	0.51	0.38	0.38	0.38
Methionine	3.1	3.1	2.2	2.2	2.2
Methionine + cystine	6.4	6.3	5.0	5.0	5.0
Threonine	6.4	6.4	4.5	4.5	4.5
Valine	8.8	8.8	6.4	6.4	6.4
Isoleucine	6.2	6.2	4.5	4.5	4.5
Tryptophan	2.2	2.2	1.7	1.7	1.7

[1]Lactation diets commenced 109.9 ± 0.1 d of gestation and fed 3 kg/d then continued *ad libitum* during lactation until weaning at 19.8 ± 0.1 d. Lactation diets continued to be fed postweaning at 2.7 kg/d until mating. Gestation diets commenced d 2 of gestation and fed 2.5 kg/d once a day for approximately 28 d. Thereafter all sows were fed 2.5 kg/d of a commercial gestation diet until farrowing.

[2]Tallow supplied by Biodiesel Producers, Barnawartha, VIC.

[3]Fish oil supplied by Optigen Ingredients®, Port Adelaide, SA.

[4]Premix provided the following nutrients (per kg diet as fed): copper, 20 mg; iron, 80 mg; organic iron, 50 mg; manganese, 40 mg; zinc, 100 mg; iodine, 1 mg; selenium inorganic, 0.15 mg; organic selenium, 0.15 mg; chromium picolinate, 3.2 mg, manganese, 40 mg; betaine, 2.5 g; antioxidant (Endox), 100 mg; vitamin A (retinol), 15,000 I.U.; vitamin D (cholecalciferol), 1,500 I.U.; vitamin E (α-tocopherol), 120 mg; vitamin B_2 (riboflavin), 3.5 mg; vitamin B_6 (pyridoxine), 2 mg; vitamin B_{12} (cyanocobalamin), 0.02 mg; biotin, 0.2 mg; folic acid, 0.5 mg; niacin, 65 mg; pantothenic acid, 5 mg.

[5]Crude fat determined by chemical analysis was 88.0 ± 3.1g/kg, Control lactation diet and 91.6 ± 2.0 g/kg, Omega-3 lactation diet; and 38.6 ± 0.9 g/kg, Control gestation and 39.8 ± 0.6 g/kg, Omega-3 gestation diet.

Table 7.2 Fatty acid composition of treatment diets offered to sows during lactation (g/100 g total fatty acids as fed basis)[1]

	Lactation		Gestation	
Fatty acid (Common name)	Control	Omega-3[1]	Control	Omega-3[2]
C16:0 (palmitic acid)	23.1	22.8	20.8	19.2
C16:1 (palmitoleic acid)	2.49	2.42	1.16	1.51
C18:0 (stearic acid)	16.1	16.1	7.5	5.5
C18:1 (oleic acid)	32.7	32.6	23.8	22.2
C18:2 n-6 (α-linoleic acid)	14.4	13.7	38.7	39.2
C18:3 n-6 (γ-linolenic acid)	0.03	0.03	0.01	0.03
C18:3 n-3 (α-linolenic)	1.60	1.59	2.89	3.18
C20:4 n-6 (arachidonic acid)	0.14	0.14	0.07	0.12
C20:5 n-3 (eicosapentaenoic acid)	0.03	0.20	0.02	0.72
C22:5 n-3 (docosapentaenoic acid)	0.11	0.17	0.05	0.30
C22:6 n-3 (docosahexaenoic acid)	0.04	0.22	0.04	0.79
Total n-6	14.7	14.1	39.0	39.6
Total n-3	1.90	2.31	3.05	5.08
n-6:n3 ratio	7.73	6.13	12.79	7.79
Total saturated	43.7	43.5	31.1	27.6
Total transaturated	2.96	3.02	0.82	0.90

[1]Fish oil included at 3 g/kg diet. [2]Fish oil included at 6 g/kg diet.

Table 7.3 Resumption of oestrous and weaning to oestrus interval of sows fed a lactation diet prefarrowing and during lactation either unsupplemented (Control) or supplemented with omega-3 PUFA as 3 g fish oil/kg of diet (Omega-3)

	Treatment[1]		
Item	Control lactation[2]	Omega-3 lactation[2]	*P* value
Number sows weaned	565	571	
Wean to oestrus, d[3]	6.8 ± 0.3	7.2 ± 0.3	0.268
Number sows mated	544	546	
Sows mated, % weaned	96.3	95.6	χ^2 0.32, 0.572

[1]Lactation diets commenced 112.1 ± 0.1 d of gestation and fed 3 kg/d then continued *ad libitum* during lactation until weaning at 19.8 ± 0.1 d. Lactation diets continued to be fed postweaning at 2.7 kg/d until mating. Gestation diets commenced d 2 of gestation and fed 2.5 kg/d once a day for approximately 28 d. Thereafter all sows were fed 2.5 kg/d of a commercial gestation diet until farrowing.
[2]At commencement of lactation treatment feeding, No. of Control sows = 608, No. of Omega-3 sows = 608
[3]Lactation length (19.8 ± 0.1 d) included in model as covariate factor (19.8 days).

Table 7.4 Subsequent farrowing rate and litter size born of sows following being fed either a Control or Omega-3 supplemented lactation diet prefarrowing, during lactation and postweaning and a Control or Omega-3 supplemented gestation diet during early pregnancy

	Treatment[1]				
	Control lactation		Omega-3 lactation		
Item	Control gestation	Omega-3 gestation	Control gestation	Omega-3 gestation	*P* value
Number sows mated	208	190	233	229	
Number sows farrowed	173	150	199	193	
Farrow rate, %	83.2	78.9	85.4	84.3	χ^2 3.45, 0.327
Subsequent number born live[2]	10.6 ± 0.3	11.4 ± 0.3	10.8 ± 0.2	11.1 ± 0.2	0.234
Subsequent number still born[3]	1.1 ± 0.1	1.2 ± 0.1	1.2 ± 0.1	1.5 ± 0.1	0.100
Subsequent number total born[4]	11.7 ± 0.3[a]	12.6 ± 0.3[b]	12.0 ± 0.2[ab]	12.6 ± 0.2[b]	0.041

[1]Lactation diets commenced 109.9 ± 0.1 d of gestation and fed 3 kg/d then continued *ad libitum* during lactation until weaning at 19.8 ± 0.1 d. Lactation diets continued to be fed postweaning at 2.7 kg/d until mating. Gestation diets commenced d 2 of gestation and fed 2.5 kg/d once a day until approximately 28 d of gestation.
[2]Estimated marginal mean values with weaning age (19.8 ± 0.1 d) and average lifetime born live (11.4 ± 0.1) included as covariate factors.
[3]Estimated marginal mean values with weaning age (19.8 ± 0.1 d), average lifetime still born (0.8 ± 0.2) included as covariate factors.
[4]Estimated marginal mean values with weaning age (19.8 ± 0.1 d), average lifetime total born (12.2 ± 0.1) included as covariate factors.
[ab]Estimated marginal mean values ± SE within row with different superscripts are significant $P < 0.05$.

Table 7.5 Subsequent litter size total born within weaned parity 1, parity 2 – 3 and old sows (parity 4 – 7) fed either Control or Omega-3 lactation diets prefarrowing, during lactation and postweaning and Control or Omega-3 gestation diets fed during early pregnancy

	Treatment[1]				
	Control lactation		Omega-3 lactation		
Item	Control gestation	Omega-3 gestation	Control gestation	Omega-3 gestation	*P* value
Parity 1,(number sows)	(21)	(16)	(22)	(16)	
Subsequent number born live[2]	9.8 ± 0.9	12.1 ± 0.8	11.5 ± 0.7	11.2 ± 0.8	0.298
Subsequent number total born[3]	11.5 ± 1.0	12.6 ± 0.9	12.4 ± 0.7	11.6 ± 0.9	0.769
Parity 2 – 3, (number sows)	(74)	(60)	(88)	(80)	
Subsequent number born live[4]	12.0 ± 0.32	11.4 ± 0.40	11.6 ± 0.30	11.7 ± 0.31	0.696
Subsequent number total born[5]	12.9 ± 0.31	12.8 ± 0.36	12.5 ± 0.29	12.8 ± 0.30	0.771
Parity 4 – 7, (number sows)	(78)	(74)	(89)	(97)	
Subsequent number born live[6]	10.0±0.39	11.1±0.41	10.3±0.37	10.7±0.06	0.226
Subsequent number total born[7]	11.1±0.40[a]	12.4±0.42[bc]	11.7±0.38[ab]	12.8±0.37[c]	0.012

[1]Lactation diets commenced 109.9 ± 0.1 d of gestation and fed 3 kg/d then continued *ad libitum* during lactation until weaning at 19.8 ± 0.1 d. Lactation diets continued to be fed postweaning at 2.7 kg/d until mating. Gestation diets commenced d 2 of gestation and fed 2.5 kg/d once a day until approximately 28 d of gestation.
[2] Estimated marginal mean values with weaning age (18.7 ± 0.2 d) and average lifetime born live (10.4 ± 0.1) included as covariate factors.
[3]Estimated marginal mean values with weaning age (18.7 ± 0.2 d), average lifetime total born (11.3 ± 0.1) included as covariate factors.
[4]Estimated marginal mean values with weaning age (20.3 ± 0.1 d), average lifetime born live (11.4 ± 0.1) included as covariate factors
[5]Estimated marginal mean values with weaning age (20.3 ± 0.2 d), average lifetime total born (12.2 ± 0.1) included as covariate factors.
[6]Estimated marginal mean values with weaning age (19.7 ± 0.1 d), average lifetime born live (11.6 ± 0.1) included as covariate factors
[7]Estimated marginal mean values with weaning age (19.7 ± 0.1 d), average lifetime total born (12.5 ± 0.1) included as covariate factors
[abc]Estimated marginal mean values ± SE within row with different superscripts are significant $P < 0.05$.

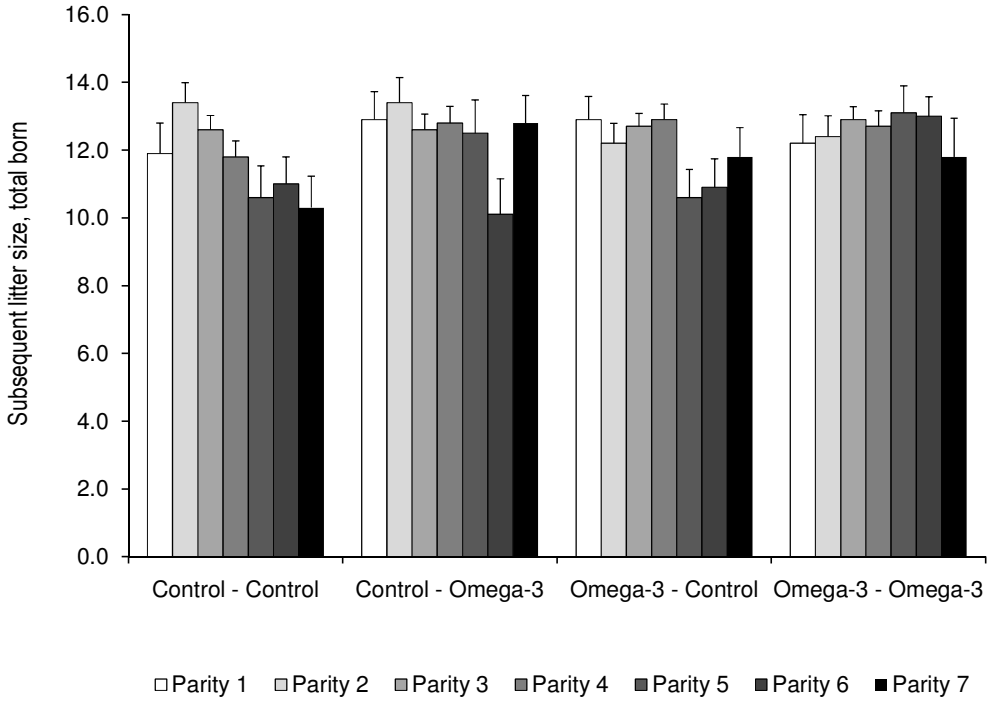

Figure 7.2 Subsequent litter size (total born) x parity at weaning (mean ± SE) when sows are fed combinations of unsupplemented (Control) or omega-3 diets containing fish oil (Omega-3) either during lactation, postweaning and/or early pregnancy

[1]Lactation diets commenced 109.9 ± 0.1 d of gestation and fed 3 kg/d then continued *ad libitum* during lactation until weaning at 19.8 ± 0.1 d. Lactation diets continued to be fed postweaning at 2.7 kg/d until mating. Gestation diets commenced d 2 of gestation and fed 2.5 kg/d once a day until approximately 28 d of gestation.

7.4 Discussion

In Chapter 3, litter size was increased subsequent to feeding multiparous sows a diet supplemented with omega-3 PUFA from fish oil prefarrowing and during lactation, and in Chapter 6 this was associated with an increase in embryo survival when supplementation continued postweaning up to mating. The aim of the present study was to evaluate reproductive performance when diets were continued after mating for the first four weeks of gestation and to determine if there is a specific nutritional window either before or after mating when the response to omega-3 supplementation is maximal. Feeding sows with fish oil diets during lactation, postweaning and continuing through to four weeks of pregnancy increased subsequent litter size by 0.9 pig total born. In younger parities there was little difference between omega-3 supplemented sows and unsupplemented sows in subsequent litter size. The largest response was observed in older parities (4 – 7 at weaning) with subsequent litter size total born increasing by 1.7 piglets when omega-3 supplementation was continued after mating for 28 d gestation. Together, these findings support the hypothesis that feeding diets with long-chain omega-3 PUFA as fish oil during lactation and continuing through to early pregnancy is beneficial in older sows.

In previous studies the response to omega-3 PUFA fed as fish products in sows has been equivocal with some reporting positive responses on litter size born (Palmer et al., 1970; Webel et al., 2004), whereas other studies reported no significant increase in litter size born (Rooke et al., 2001c; Mateo et al., 2009); embryo number or embryo survival in weaned mixed parity sows (Perez-Rigau et al., 1995); or in gilts (Estienne et al., 2006). In Chapter 3, we reported an increase in subsequent litter size (live born and total born) when diets containing fish oil were fed 8 d prefarrowing and during lactation for 19 d. In Chapter 4, we failed to demonstrate an increase in litter size born when gilts were fed supplemented omega-3 diets during puberty and up to mating.

Although subsequent total born was increased in sows fed omega-3 supplemented diets, subsequent live born and farrowing rate were not. Total litter size born is a factor of both live born and still born, the latter increasing with parity (Dial et al., 1992). Still births are generally caused by anoxia and stress during prolonged parturition. Placental efficiency and/or uterine capacity can also contribute to perinatal losses (Vonnahme et al., 2002; Wilson, 2007) including mummified piglets. As total litter size increased in the present study, so did the tendency for an increase in still births and mummified foetuses ($P < 0.100$). Nevertheless, the increase in subsequent total born in supplemented sows, particularly those fed the omega-3 diet in gestation, was reflected in a trend for increase in subsequent live born of up to 0.8 piglets over all parities and 1.1 piglets live born in older sows. A larger sample size may be required to demonstrate benefits of supplementation on subsequent live born due to antagonistic effects on still births, particularly in older sows. The proportion of sows mated after weaning, wean to oestrus interval, and farrowing rate are all important reproductive outcomes that influence the productivity of the breeding herd. These were unaffected by dietary supplementation, supporting earlier results reported in this thesis in sows (Chapter 3 and 6).

Low reproductive performance has been recognized as a major contributor to high sow culling rates and early exit from the breeding herd. Hughes and Varley (2003) illustrated from commercial records where this study was conducted, that sow litter size declines after parity 4. In the past, sow fecundity remained high beyond parity 4 or 5, such that sows were not culled for age due to declining litter size and increased still births until parity 7 or older. Increasing the fertility of multiparous sows and maintaining maximum fertility levels for longer increases sow longevity and lifetime performance, and consequently profitability from the breeding herd (Dijkhuizen et al., 1989; Dhuyvetter, 2000; Levis, 2005). Depending on costs, the industry regards that culling sows before parity 3 is below the financial break-even return for gilt replacements (Levis, 2005). Therefore, this outcome may provide a nutritional solution for producers to keep sows at a high level of productivity for longer.

In the present study, the response in subsequent litter size total born in parity 4 – 7 sows was maximal when supplemented diets were fed for four weeks before and continued four weeks after mating (15% higher than Control – Control group). Feeding fish oil diets after mating from d 1 to previously unsupplemented sows produced a similar response (11% higher than Control – Control group). However, there was a smaller response when omega-3 diets were fed for four weeks up to mating but not in gestation (5% higher than Control – Control group). This was reflected in similar increases in the number live born.

The aim of the study was to identify when the response in litter size and farrowing rate occurs and to identify possible mechanisms for the response. The combined results from Chapter 3 and 6 suggest that supplementation with omega-3 PUFA prior to ovulation increases subsequent litter size born due to an increase in embryo survival. In Chapter 6 we proposed that embryo survival may increase with omega-3 PUFA supplementation due to improved oocyte quality and developmental competence and/or an improved environment for implantation due to altered prostaglandin and progesterone metabolism. In the present study, sows that were fed a supplemented diet from the d 1 after mating continuing for four weeks of gestation produced a similar litter size as those supplemented from 3 d before farrowing up to mating. This suggests that there may be more than one mechanism by which omega-3 PUFA affects reproduction performance: one which affects oocyte developmental competence; and another which affects implantation and luteal support of the developing embryo. The relative importance of either mechanism is not clear from the present study, with only a small additive effect of supplementation before mating and during early gestation compared to the litter size response to omega-3 PUFA either before mating or after mating alone. Omega-3 PUFA has been reported to improve in-vitro oocyte developmental competence in sheep (Zeron et al., 2002) and from our own studies in sows (Mitchell et al., 2010). Although in Chapter 6 it was shown that omega-3 PUFA supplementation before mating improved embryo survival in older parity sows, the

effect of feeding supplemented diets after ovulation on embryo survival has not been investigated and forms the basis for the following chapter.

In conclusion, feeding sows with diets supplemented with fish oil prefarrowing, lactation and continuing to mating and/or early pregnancy for four weeks of gestation increased subsequent litter size, with a larger response observed in older parity sows than younger sows. Feeding sows omega-3 PUFA as fish oil during this time therefore offers producers a nutritional strategy that could overcome declining productivity in sows increasing in age. Furthermore, this finding confirms that omega-3 PUFA influences embryo survival in these parities. Possible mechanisms whereby this response is mediated will be examined in the following chapters.

Chapter 8 The effect of omega-3 PUFA supplementation fed prior to mating and in early gestation on embryo survival and peri-implantation progesterone level in sows

8.1 Introduction

The experiments described in Chapters 3, 6 and 7 demonstrated that adding omega-3 PUFA as fish oil increased litter size in sows, particularly those from older parities. The mechanism(s) responsible for this improvement is unknown. In Chapter 6, supplementation of omega-3 PUFA increased embryo survival and embryo number at 23 d of gestation but had no effect on ovulation rate when sows were fed from 6 d prefarrowing through to mating. In these studies, there was no evidence of a dietary effect on energy intake, litter growth rate during lactation or sow live weight and P2 loss. Brazle et al. (2009) reported that changes in dietary supply of EPA and DHA alters the concentration of long-chain omega-3 PUFA in uterine tissue and the conceptus, as gilts fed diets with high EPA showed an increase in EPA in the endometrium of the uterus, and both EPA and DHA were increased in the embryo and surrounding tissue around the time of implantation (Brazle et al., 2009). Long-chain PUFA have an important role in regulating reproduction through the eicosanoid pathway which synthesises prostaglandins (Caughey et al., 2005). The pig oocyte and follicle contains relatively high amounts of PUFA compared to other species, particularly the omega-6 acids LIN and ARA which are involved in prostaglandin synthesis (Khandoker et al., 1997; McEvoy et al., 2000). However, it is not known if omega-3 fatty acids in sow diets supplemented with fish oil have a role in prostaglandin or progesterone synthesis.

In Chapter 7, the effect of omega-3 PUFA from diets containing fish oil fed either for four weeks prior to mating and/or the first four weeks of gestation was investigated. Feeding the sow a supplemented

diet after mating following a period of no supplementation prior to mating resulted in a higher subsequent litter size born compared to unsupplemented sows throughout. Furthermore there was an additive effect when supplementation before mating was combined with supplementation after mating for up to four weeks of gestation. The effect of omega-3 PUFA on embryo survival when supplemented after mating for four weeks has not been previously reported in pigs. During this period up to 40% of embryo loss occurs and has a significant contribution on potential litter size born (Foxcroft et al., 2006). Increasing dietary EPA and DHA has been associated with a tendency for proportionately more medium and large follicles in dairy cows (Petit et al., 2002) and in sows (Mitchell et al., 2010). An enhanced follicle growth and development may increase the functionality of the subsequent corpora lutea (CL) and progesterone synthesis (Smith et al., 1994). Continuing to feed diets high in EPA and DHA in early gestation may also have an anti-luteolytic effect reducing synthesis of prostaglandin $F_{2\alpha}$ from the omega-6 precursor ARA and thereby maintain progesterone levels during peri-implantation and early pregnancy (Mattos et al., 2004).

The aim of this present study was to determine if the higher litter size born observed in Chapter 7 by feeding omega-3 PUFA supplemented diets pre- and postmating was due to additive effects on embryo survival in multiparous sows. Secondly, we examined whether supplementation increases progesterone production around the time of implantation, thereby identifying a possible mechanism for improved litter size in sows fed omega-3 PUFA as fish oil.

8.2 Materials and Methods

8.2.1 Animals and diets

The experiment was conducted during summer (December to February). Over five weeks, 133 Large White x Landrace F1 sows were allocated to the experiment at 109.9 ± 0.1 d of pregnancy

(mean ± SE). Sows ranged in parity before farrowing from 3 to 6, with an average of 4.56 ± 0.10. Diet composition was similar to those used in Chapter 7 with the exception of the removal of zinc bacitracin from the lactation diets and seasonal variation in nutrient content of cereal grains (Table 8.1). Sows were allocated based on parity and randomized for previous litter size born to either a lactation diet with no added fish oil (Control Lactation) or a diet containing fish oil added at 3 g/kg diet as a partial replacement of tallow (Omega-3 Lactation). Sows were fed their respective lactation diets 6.7 ± 0.2 d prefarrowing and during lactation until weaning at 26.5 ± 0.2 d after farrowing. After weaning sows were moved to individual stalls and continued to be fed their respective lactation diet up to mating on the first oestrus after weaning. From d 2 of gestation (2 days after first AI), sows within each lactation dietary regimen were fed either a treatment gestation diet with no added fish oil (Control Gestation) or a diet containing fish oil added at 6 g/kg diet (Omega-3 Gestation). The higher level was provided to keep the daily intake of EPA + DHA at the recommended level of 2.8 g. Sows remained on their gestation diets until slaughter at 22.9 ± 0.1 d. The experiment was designed as a 2 x 2 factorial with the following treatment feeding regimens: sows fed a lactation diet and gestation diet without fish oil (Control – Control); sows fed a lactation diet with fish oil and a gestation diet without fish oil (Omega-3 – Control); sows fed a lactation diet without fish oil and a gestation diet with fish oil (Control – Omega-3); sows fed a lactation and gestation diet with fish oil (Omega-3 – Omega-3). The treatment feeding regimen has been previously described in Chapter 7. Treatment lactation diets and treatment gestation diets were formulated to be isocaloric (Table 8.1). Diets were sampled and 200 g per treatment diet were stored at -20^{o}C for dietary fatty acid analysis at the Nutrition and Functional Foods Laboratory, The University of Adelaide, Waite Campus, SA (Table 8.2). A full description of the dietary fatty acid analytical technique is provided in Chapter 2.

8.2.2 Feeding and animal management

On entry to the farrowing shed, 67 sows were allocated to the unsupplemented lactation diet (Control) and 66 sows to the Omega-3 lactation diet. Sows were individually housed in farrowing

crates and fed 3 kg once a day (0700 h) of their lactation diet until farrowing. Farrowing date, litter size born live and still born, including mummified foetuses, were recorded. Data on previous litter size born live and total born (including mummified foetuses and stillborns) was retrieved from farm records and combined with current litter size records as a lifetime average total born record. Litter size was adjusted within treatment according to rearing ability and ranged in number from 8 to 14. From the day after farrowing, sows were offered their treatment diet up to three times a day based on appetite. Sow lactation intake and piglet and litter weight gain during lactation was not recorded. Once a week, sows were weaned from their litters and relocated to individual gestation housing. Litter size weaned and weaning age were recorded. Sows continued to be fed their respective lactation diet at 2.7 kg once a day (0700 h) until mated by AI on first postweaning oestrus in an am/am service, 24 h apart. Due to space limitations, sows that failed to show oestrus within 7 d were recorded as anoestrus for the purposes of this expeiment, removed from their treatment diet and relocated elsewhere in the shed and took no further part in the study. Once mated, sows were allocated to their gestation diet treatment and fed 2.5 kg once a day (0700 h) from d 2 (day after the second AI) until slaughter at a commercial abattoir 22.9 ± 1.1 d postmating. Approximately 30 min after exsanguination, reproductive tracts were recovered for each animal. Ovaries were examined and ovulation rate determined as the sum total of corpora lutea on both ovaries. Uteri were dissected and visible embryos were emptied onto a dissection tray and the total number of recovered embryos counted.

8.2.3 Blood collection and analysis

Blood samples were collected from all sows by jugular venipuncture at 1130 h, approximately 5 h after feeding on d 10, 12 and 14 of gestation. Further details are provided in Chapter 2. Blood samples were collected on ice in lithium heparin tubes and centrifuged at 2000 g for 5 min. Plasma was drawn off with a disposable pipette and collected into a separate 5 ml tube and stored at -20^{o}C. On confirmation of pregnancy, samples from nine sows per treatment sampled at d 10 and d 14 were

analysed for total fatty acids at the Nutrition and Functional Foods Laboratory, The University of Adelaide, Waite Campus, SA. The method is described in Chapter 2. Where a complete set of samples on d 10, 12 and 14 of gestation were available from pregnant sows, these were analyzed for progesterone at the Obstetrics and Gynaecology Department, Medical School, The University of Adelaide, Adelaide, SA. Samples were prepared in duplicate by radioimmunoassay in 50 μL of a 1:10 dilution of plasma in duplicate according to the manufacturers instructions (IM1188; Beckman Coulter, Brea, CA, USA). The lowest detectable concentration was 0.5 pg/tube (equivalent of 1 ng/mL). The intra assay coefficient of variation was less than 10% and the inter assay coefficient of variation was less than 15% at 57 pg/tube (114 ng/mL).

8.2.4 Statistical analysis

Data was processed through a General Linear Model (GLM) analysis of variance for treatments in a randomized design model assuming equal variance (Sokal and Rohlf, 1981). The analysis of variance was performed using SPSS v 18.0 (SPSS Inc., 1989-2009). Ovulation rate, reported as the sum of corpora lutea on both ovaries, and embryos recovered were normally distributed and analyzed by GLM Univariate ANOVA. Replicate and sow parity at the start of the experiment were included as random factors in the model. Lifetime average total born and weaning age were included as covariate factors. Pairwise comparisons for each dependent variable between treatments were performed by Least Significance Difference test and compared at $P < 0.05$. The proportion of weaned sows that were mated within 7 d and the proportion pregnant at slaughter were analyzed by chi square. Concentrations of fatty acid and progesterone in plasma were analyzed as repeated measures with gestation age and diet treatment included in the model and assessed for significance as main effects. Probability values < 0.05 were described as significant, and $P < 0.10$ were used to describe trends.

8.3 Results

8.3.1 Diet fatty acids

The level of crude fat analyzed was considerably higher than formulated in the gestation diets (Table 8.1). There was a three-fold increase in EPA and DHA with the addition of 3 g fish oil/kg diet to the lactation diet, and the addition of 6 g fish oil/kg diet resulted in an 11-fold increase in the gestation diet compared to the Control lactation and gestation diets, respectively (Table 8.2). There was no significant difference in crude fat content between Omega-3 and Control lactation or gestation diets. Based on the analyzed crude fat levels, the calculated sum of EPA + DHA was 0.28 g/kg diet in the Omega-3 lactation diet and 0.09 g/kg diet in the Control lactation diet. The sum of EPA + DHA was calculated as 0.47 g/kg diet in the Omega-3 gestation diet and 0.04 g/kg diet in the Control gestation diet (Table 8.2). Based on the daily feeding level of 2.5 kg in gestation, the Omega-3 gestation diet provided 1.18 g/d EPA and DHA, below the daily recommendation of 2.8 g/d proposed by Leskanich and Noble (1999), whilst the Control gestation diet provided 0.1 g/d EPA and DHA. Because lactation feed intake was not measured in the present study, the daily intake of EPA and DHA from lactation diets during lactation was not determined.

8.3.2 Plasma fatty acid profile

The omega-6 LIN and omega-3 ALA increased between d 10 and d 14, whilst the omega-6 ARA decreased in plasma between d 10 and d 14 (Table 8.3). The effect of gestational age over the period of peri-implantation did not change the plasma levels of the other omega-3 fatty acids, EPA and DHA, nor the total concentration of omega-6 PUFA, omega-3 PUFA or the ratio of omega-6:omega-3 (Table 8.3).

There was no effect of dietary treatment regimen on LIN, ALA or ARA, however, dietary treatment significantly altered plasma levels of EPA, DHA and total omega-3 and omega-6:omega-3 ratio (Table 8.3). Although the dietary addition of EPA + DHA in the gestation Omega-3 diet was lower

than recommended, there was a 2.7-fold increase in plasma EPA + DHA in sows on Control – Omega-3 feeding regimen and a 3.3-fold increase in sows on Omega-3 – Omega-3 regimen. The treatment responses on d 10 and 14 for EPA and DHA, total omega-3, and the omega-6:omega-3 ratio were compared as average values over the 10 – 14 d sampling period (Figure 8.1). Plasma EPA from Control – Control sows and Omega-3 – Control sows were significantly lower ($P < 0.01$) compared to Control – Omega-3 and Omega-3 – Omega-3 sows (Figure 8.1a), whilst DHA was significantly different ($P < 0.01$) between each of the four dietary regimens (Figure 8.1b). Although total omega-6 plasma levels were unaffected by diet (Table 8.3), total omega-3 plasma levels were affected (Figure 8.1c). The total omega-3 level was higher ($P < 0.01$) in sows on Omega-3 – Omega-3 compared to sows on Control – Omega-3 regimen, which were higher than either Control – Control sows or Omega-3 – Control sows. Consequently, the ratio of omega-6:omega-3 fatty acids differed significantly ($P < 0.01$) between sows such that those on Control – Control > Omega-3 – Control > Control – Omega-3 and Omega-3 – Omega-3, the latter two treatments being similar (Figure 8.1d).

8.3.3 Reproductive performance postweaning and early pregnancy

The litter size weaned was unaffected by dietary treatment during lactation and averaged 9.7 ± 0.1 pigs per sow. Litter weight gain and average piglet weaning weight were not recorded. Of the 133 sows allocated to the study, 128 sows were mated within seven days of weaning. There were two sows on the Control lactation diet and three sows on the Omega-3 lactation diet that failed to resume oestrous within seven days. Feeding the Omega-3 lactation diet during lactation and postweaning did not affect the proportion of sows mated (65/67 sows allocated to Control lactation vs 63/66 sows allocated to Omega-3 lactation; χ^2 0.22; $P = 0.636$), or the weaning to oestrus interval (4.7 ± 0.1 d vs 4.6 ± 0.1 d; $P = 0.193$).

The number of sows within each of the four treatments following mating was 34, 30, 29 and 34 for Control – Control, Control – Omega-3, Omega-3 – Control and Omega-3 – Omega-3 regimens, respectively. Two mated sows (1 x Omega-3 – Control, 1 x Control – Omega-3) were condemned at the abattoir and the reproductive tract was not recovered, so no assessment on pregnancy was possible. One Omega-3 – Omega-3 sow was euthanased two days after mating due to a leg injury.

The proportion of sows confirmed to be pregnant at slaughter did not differ significantly between treatments (Table 8.4). Ovulation rate was also unaffected by treatment. Diet had a significant effect on the number of embryos recovered, with a higher number of embryos in Omega-3 sows compared with unsupplemented sows ($P < 0.05$; Table 8.4). The number of embryos recovered was similar between sows on the diet combinations of Control – Omega-3; Omega-3 – Control; and Omega-3 – Omega-3; and these were significantly different to Control – Control sows ($P < 0.05$). Embryo survival tended ($P < 0.10$) to be affected by dietary treatment (Table 8.4), such that Control – Control sows had a lower ($P < 0.05$) embryo survival compared to sows fed combinations of omega-3 PUFA (Control – Omega-3; Omega-3 – Control; Omega-3 – Omega-3) when analyzed by pairwise comparison.

8.3.4 Plasma progesterone response to dietary treatment and time of gestation

Dietary treatment regimen had no effect on plasma progesterone sampled on d 10, d 12 and d 14 of gestation (Table 8.5). Over the three sampling periods covering peri-implantation, plasma progesterone averaged 21.7 ± 1.7 ng/mL for Control – Control; 22.5 ± 1.2 ng/mL for Control – Omega-3; 22.5 ± 1.2 ng/mL for Omega-3 – Control; and 21.1 ± 1.1 ng/mL for Omega-3 – Omega-3 ($P = 0.786$).

Plasma progesterone was significantly affected by day of gestation when sampled ($P < 0.01$) with progesterone increasing on d 12 of gestation (23.8 ± 0.8 ng/mL) compared to d 10 (20.4 ± 0.8 ng/mL) and d 14 (21.5 ± 0.6 ng/mL). The progesterone concentration was similar on d 10 and d 14

(*P* = 0.297). There was a tendency for day of gestation x treatment regimen to affect plasma progesterone (*P* = 0.09). On d 12, progesterone levels were elevated in the omega-3 treatments, however, univariate data analysis for treatment differences were not significant (*P* = 0.461; Table 8.5).

Table 8.1 Ingredient and calculated nutritional composition of experimental lactation diets fed prefarrowing and during lactation and postweaning and experimental gestation diets fed after mating until slaughter (as fed basis)

	Lactation diet[1]		Gestation diet[1]	
Ingredient, g/kg	Control	Omega-3	Control	Omega-3
Wheat	565	565	498	498
Barley	93	93	246	246
Millrun (wheat middlings)	74	74	190	190
Canola meal	60	60	-	-
Soybean meal	40	40	-	-
Meat meal	57	57	16.7	16.7
Blood meal	10	10	-	-
Molasses	10	10	-	-
Tallow[2]	68	65	10	4
Fish oil[3]	-	3	-	6
Water	-	-	10	10
Limestone	9.0	9.0	12.3	12.3
Dicalcium phosphorus	-	-	9.0	9.0
Potassium chloride	4.2	4.2	-	-
Salt	3.5	3.5	3.0	3.0
Synthetic lysine (L-lysine HCl)	1.4	1.4	1.5	1.5
Synthetic threonine	0.2	0.2	0.2	0.2
Mineral vitamin premix[4]	2.2	2.2	2.2	2.2
Monensin	1.0	1.0	1.0	1.0
Betaine	1.0	1.0	-	-
Phytase	0.7	0.7	0.1	0.1
Xylanase	0.3	0.3	-	-
Mycotoxin binder	0.5	0.5	0.5	0.5
Antioxidant	0.2	0.2	-	-
Calculated nutrient analyses, g/kg				
Digestible energy (MJ/kg)	14.9	14.9	13.0	13.0
Crude protein	184	184	140	140
Crude fat[5]	85	85	27	27
Crude fibre	36	36	42	42
Calcium	9.1	9.1	9.3	9.3
Total phosphorus	5.6	5.6	6.5	6.5
Available phosphorus	4.5	4.5	4.9	4.9
Lysine	9.0	9.0	5.9	5.9
Ileal digestible lysine (g/MJ DE)	0.50	0.50	0.37	0.37
Methionine	3.0	3.0	2.1	2.1
Methionine + cystine	6.1	6.1	4.8	4.8
Threonine	6.2	6.2	4.3	4.3
Valine	8.5	8.5	6.1	6.1
Isoleucine	5.8	5.8	4.2	4.2
Tryptophan	2.1	2.1	1.7	1.7

[1]Lactation diets commenced 109.9 ± 0.1 d of gestation and fed 3 kg/d then continued *ad libitum* during lactation until weaning at 26.5 ± 0.2 d. Lactation diets continued to be fed postweaning at 2.7 kg/d until mating. Gestation diets commenced d 2 of gestation and were fed 2.5 kg/d once a day until slaughter at 22.9 ± 1.1 d of gestation.

[2]Tallow supplied by Biodiesel Producers, Barnawartha, VIC.

[3]Fish oil supplied by Optigen Ingredients®, Port Adelaide, SA.

[4]Premix provided the following nutrients (per kg diet as fed): copper, 20 mg; iron, 80 mg; organic iron, 50 mg; manganese, 40 mg; zinc, 100 mg; iodine, 1 mg; selenium inorganic, 0.15 mg; organic selenium, 0.15 mg; chromium picolinate, 3.2 mg, manganese, 40 mg; betaine, 2.5 g; antioxidant (Endox), 100 mg; vitamin A (retinol), 15,000 I.U.; vitamin D (cholecalciferol), 1,500 I.U.; vitamin E (α-tocopherol), 120 mg; vitamin B_2 (riboflavin), 3.5 mg; vitamin B_6 (pyridoxine), 2 mg; vitamin B_{12} (cyanocobalamin), 0.02 mg; biotin, 0.2 mg; folic acid, 0.5 mg; niacin, 65 mg; pantothenic acid, 5 mg.

[5]Crude fat determined by chemical analysis was 88.0 ± 3.1 g/kg, Control lactation diet and 91.6 ± 2.0 g/kg, Omega-3 lactation diet; and 38.6 ± 0.9 g/kg, Control gestation and 39.8 ± 0.6 g/kg, Omega-3 gestation diet.

Table 8.2 Fatty acid composition of treatment diets offered to sows during lactation (g/100 g total fatty acids as fed basis)

	Lactation diet[1]		Gestation diet[1]	
Fatty acid (Common name)	Control	Omega-3[2]	Control	Omega-3[3]
C16:0 (palmitic acid)	23.0	23.2	20.7	20.0
C16:1 (palmitoleic acid)	2.83	2.84	1.33	1.66
C18:0 (stearic acid)	14.7	14.8	7.0	6.0
C18:1 (oleic acid)	32.6	32.3	22.1	23.4
C18:2 n-6 (α-linoleic acid)	14.1	13.9	39.0	37.2
C18:3 n-6 (γ-linolenic acid)	0.04	0.03	0.03	0.03
C18:3 n-3 (α-linolenic)	1.55	1.54	3.39	3.53
C20:4 n-6 (arachidonic acid)	0.15	0.16	0.10	0.13
C20:5 n-3 (eicosapentaenoic acid)	0.03	0.10	0.02	0.50
C22:5 n-3 (docosapentaenoic acid)	0.12	0.16	0.07	0.28
C22:6 n-3 (docosahexaenoic acid)	0.06	0.17	0.05	0.67
Total n-6	14.5	14.3	39.4	37.6
Total n-3	2.41	2.62	3.80	5.24
n-6:n3 ratio	6.02	5.44	10.35	7.18
Total saturated	42.5	42.8	30.6	28.9
Total transaturated	2.94	2.92	1.31	1.00

[1]Lactation diets commenced 109.9 ± 0.1 d of gestation and fed 3 kg/d then continued *ad libitum* during lactation until weaning at 26.5 ± 0.2 d. Lactation diets continued to be fed postweaning at 2.7 kg/d until mating. Gestation diets commenced d 2 of gestation and were fed 2.5 kg/d once a day until slaughter at 22.9 ± 1.1 d of gestation.
[2]Fish oil included in the lactation diet at 3 g/kg diet.
[3]Fish oil included in the gestation diet at 6 g/kg diet.

Table 8.3 Fatty acid composition of plasma (g/100 g total fatty acids) on d 10 and 14 gestation taken from sows (n = 9 per treatment) fed either unsupplemented (Control) or Omega-3 lactation diet prefarrowing, during lactation and postweaning and unsupplemented (Control) or Omega-3 gestation diet during early pregnancy

	Treatment[1]										
	Control lactation				Omega-3 lactation				Main effects		
Item	Control gestation		Omega-3 gestation		Control gestation		Omega-3 gestation			*P* value	
	Day 10	Day 14	Day 10	Day 14	Day 10	Day 14	Day 10	Day 14	SED	Day	Treat.
C18:2 n-6 (LIN)	22.1	23.8	20.9	21.1	21.7	23.6	23.5	23.8	0.3	0.036	0.138
C18:3 n-3 (ALA)	0.67	0.82	0.66	0.76	0.74	0.82	0.81	0.91	0.02	0.012	0.284
C20:4 n-6 (ARA)	7.14	6.44	6.58	6.35	6.46	6.19	6.50	5.70	0.13	0.011	0.577
C20:5 n-3 (EPA)	0.30	0.27	0.67	0.73	0.28	0.28	0.84	0.82	0.03	0.817	<0.001
C22:6 n-3 (DHA)	0.38	0.33	0.95	1.12	0.62	0.56	1.29	1.33	0.05	0.288	<0.001
Total n-6	30.4	31.5	28.5	28.6	29.1	31.0	31.0	30.5	0.36	0.271	0.154
Total n-3	2.80	2.80	3.98	4.35	3.02	3.01	4.75	4.76	0.10	0.260	<0.001
n-6:n3 ratio	10.85	11.28	7.16	6.67	9.64	10.38	6.58	6.42	0.24	0.185	<0.001

[1]Lactation diets commenced 109.9 ± 0.1 d of gestation and fed 3 kg/d then continued *ad libitum* during lactation until weaning at 26.5 ± 0.2 d. Lactation diets continued to be fed postweaning at 2.7 kg/d until mating. Gestation diets commenced d 2 of gestation and were fed 2.5 kg/d once a day until slaughter at 22.9 ± 1.1 d of gestation.

Table 8.4 Subsequent pregnancy, ovulation rate, embryo number and embryo survival for sows fed either unsupplemented (Control) or Omega-3 lactation diets prefarrowing, during lactation and postweaning and unsupplemented (Control) or Omega-3 gestation diets during early pregnancy

	Treatment[1]				
	Control lactation		Omega-3 lactation		
Item	Control gestation	Omega-3 gestation	Control gestation	Omega-3 gestation	*P* value
Number sows mated[2]	34	29	28	33	
Number sows pregnant[2]	24	25	24	28	
Pregnancy rate, % mated	70.6	86.2	85.7	84.8	χ^2 3.68, 0.298
Ovulation rate[3]	22.8 ± 0.9	24.6 ± 0.9	24.7 ± 0.9	24.3 ± 0.9	0.399
Embryos recovered[3]	14.2 ± 1.0[a]	17.6 ± 1.0[b]	17.9 ± 1.0[b]	17.5 ± 1.0[b]	0.048
Embryo survival,%[3,4]	61.4 ± 3.9	74.9 ± 3.7	74.8 ± 3.8	73.3 ± 3.8	0.061

[1] Lactation diets commenced 109.9 ± 0.1 d of gestation and fed 3 kg/d then continued *ad libitum* during lactation until weaning at 26.5 ± 0.2 d. Lactation diets continued to be fed postweaning at 2.7 kg/d until mating. Gestation diets commenced d 2 of gestation and were fed 2.5 kg/d once a day until slaughter at 22.9 ± 1.1 d of gestation.

[2]Number of sows mated where full reproductive tracts were recovered at slaughter and assessed for pregnancy.

[3]Estimated marginal mean values with weaning age (26.3 ± 0.2 d) and average lifetime total born (12.2 ± 0.2) included as covariate factors.

[4] Estimated marginal mean values with ovulation rate (23.8 ± 0.4) included as a covariate factor.

[ab]Estimated marginal mean values ± SE within column differ significantly $P < 0.05$.

Table 8.5 Mean ± SE plasma progesterone (ng/mL) sampled on different days of gestation from sows fed either unsupplemented (Control) or Omega-3 lactation diets prefarrowing, during lactation and postweaning and fed unsupplemented (Control) or Omega-3 gestation diets during early pregnancy

	Treatment				
	Control Lactation		Omega-3 Lactation		
Day of gestation	Control Gestation	Omega-3 Gestation	Control Gestation	Omega-3 Gestation	*P* value
Number sows sampled	22	22	24	26	
D 10	21.0 ± 1.6	19.6 ± 1.6	22.0 ± 1.6	18.9 ± 1.4	0.473
D 12	21.9 ± 1.6	25.0 ± 1.6	25.0 ± 1.6	23.4 ± 1.4	0.461
D 14	22.3 ± 1.3	22.8 ± 1.3	20.4 ± 1.3	20.9 ± 1.2	0.516

[1] Lactation diets commenced 109.9 ± 0.1 d of gestation and fed 3 kg/d then continued *ad libitum* during lactation until weaning at 26.5 ± 0.2 d. Lactation diets continued to be fed postweaning at 2.7 kg/d until mating. Gestation diets commenced d 2 of gestation and were fed 2.5 kg/d once a day until slaughter at 22.9 ± 1.1 d of gestation.

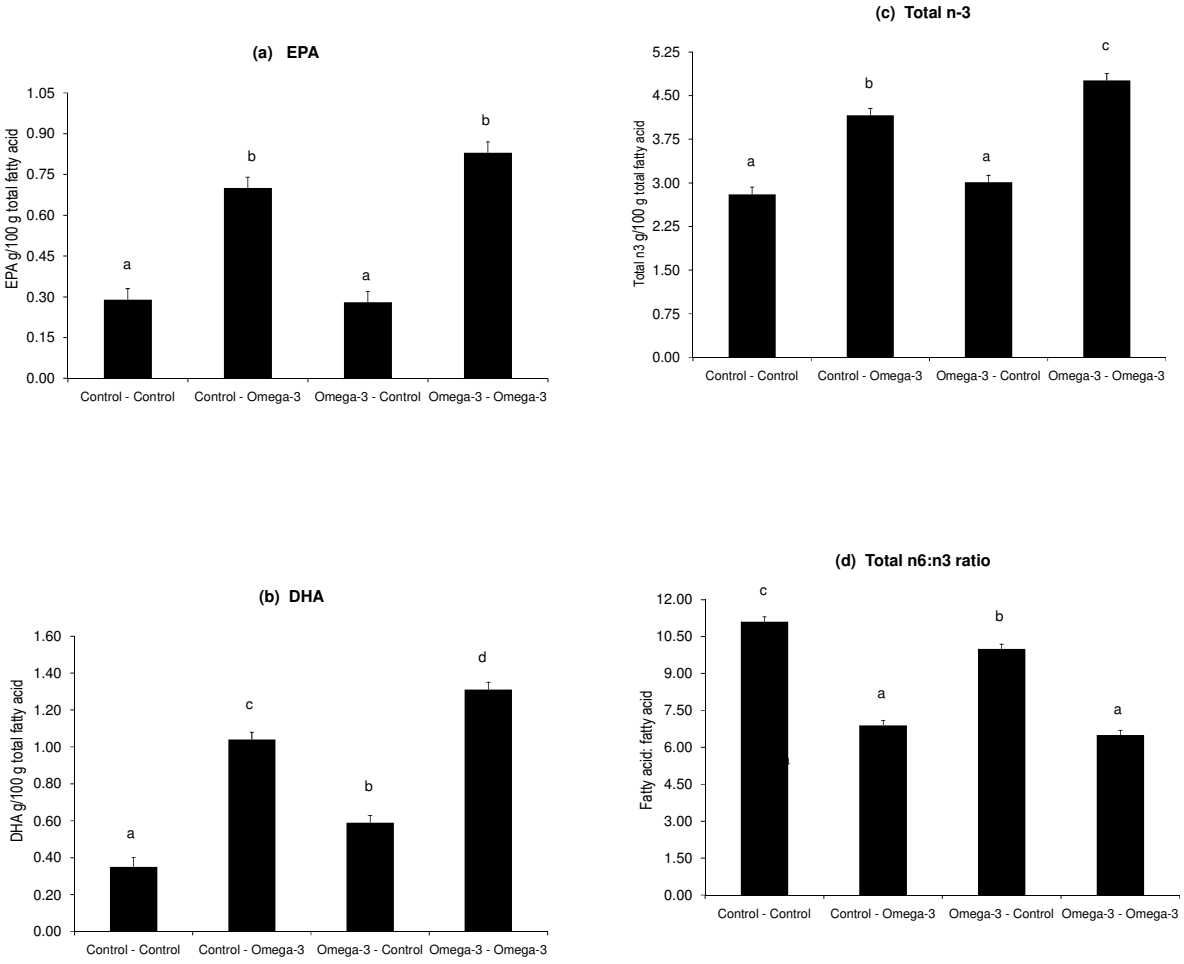

Figure 8.1 Mean ± SE plasma fatty acid levels (n = 9 per treatment) between d 10 - 14 of sows fed the dietary regimen of Control – Control, Control – Omega-3, Omega-3 – Control or Omega-3 – Omega-3[1]

[1]Lactation diets (unsupplemented Control or Omega-3) commenced 109.9 ± 0.1 d of gestation and fed 3 kg/d then continued *ad libitum* during lactation until weaning at 26.5 ± 0.2 d. Lactation diets continued to be fed postweaning at 2.7 kg/d until mating. Gestation diets (unsupplemented Control or Omega-3) commenced d 2 of gestation and were fed 2.5 kg/d once a day until slaughter at 22.9 ± 1.1 d of gestation.
[abcd]Mean values between treatments differ significantly ($P < 0.05$).

8.4 Discussion

The aim of the present study was to determine the effect on embryo survival and progesterone in high parity sows supplemented with omega-3 PUFA as fish oil after mating for the first four weeks of gestation, and to determine if additive effects of supplementation pre- and postmating occur. In Chapter 6, embryo survival was increased when older sows were fed a supplemented diet from prefarrowing up to mating without affecting ovulation rate. The results from the present study confirmed this with an increase in embryo survival of 15% in sows supplemented up to mating (Omega-3 – Control sows) compared to unsupplemented sows. This increase was similar to that reported in Chapter 6 (+14%) for corresponding dietary treatments fed to older sows (> 5th parity at weaning). The results of the present study report for the first time that feeding omega-3 PUFA as fish oil from postmating and during early pregnancy also increases embryo survival. In Chapter 7, the same dietary feeding regimen in a larger field study resulted in a 15% increase in litter size (total born) subsequent to supplementation from prefarrowing through to 28 d of gestation (Omega-3 – Omega-3) compared with unsupplemented sows (Control – Control) in parity 4 – 7 sows. This increase was reflected in the present study, with a 23% increase in the number of embryos in the Omega-3 – Omega-3 sows compared to Control – Control sows.

To further understand the mechanism whereby omega-3 PUFA improves litter size, the present study examined the effects of supplementation at two critical stages of the reproductive cycle: first when oocytes grow and mature during the antral phase of folliculogenesis; and secondly during early gestation when the majority of embryo loss occurs. In Chapter 6, feeding omega-3 diets during lactation and postweaning had no effect on ovulation rate in high parity sows, whereas embryo survival was increased. Changes to nutrition during lactation and before ovulation have been shown to affect oocyte developmental competence (Zak et al., 1997b), and consequentially embryo growth during early pregnancy (Ashworth and Antipatis, 1999; Quesnel et al., 2010). Studies in dairy cows

and sheep have reported greater follicle growth and advanced metabolism of in-vitro matured oocytes when animals were fed diets high in omega-3 PUFA (Petit et al., 2002; Zeron et al., 2002). However, there is a paucity of publications reporting on the effect of omega-3 PUFA on oocyte quality in pigs. In a preliminary study (Mitchell et al., 2010), we reported that oocytes recovered from sows fed diets supplemented with omega 3-PUFA from 8 d prefarrowing up to mating were more likely to develop to blastocyst stage in-vitro compared to unsupplemented sows. Although this finding needs to be confirmed, it does suggest that oocyte developmental competence may be one mechanism whereby embryo survival, and therefore litter size, is increased in older sows fed omega-3 diets supplemented as fish oil prior to ovulation.

The present study compared sows fed an omega-3 supplemented diet either before or after ovulation. As Control – Omega-3 sows had a higher embryo survival compared to Control – Control sows, it can be inferred that supplementation after ovulation and during early pregnancy has some role in the development of the embryo and/or the uterine environment to support the conceptus. The increase in embryo survival when sows were fed omega-3 PUFA as fish oil was similar when diets were fed either before mating or after mating. Furthermore, there was no additive effect of supplementation before and after mating as Omega-3 – Omega-3 on embryo survival in the current experiment. Unlike the present study, in the older parity sows studied in Chapter 7 there was a larger response to omega-3 supplementation postmating compared to pre-mating on subsequent litter size born (12% vs 5% increase relative to unsupplemented sows for Control – Omega-3 and Omega-3 – Control groups, respectively). This may have been due to long-term effects of omega-3 PUFA on the uterine environment, improving foetal survival later in gestation.

Progesterone is essential for the early embryo development and maintenance of pregnancy (Geisert and Yelich, 1997). In the present study, ovulation rate was not affected, so an increase in progesterone would only be possible if the output per CL was increased due to increased luteal

progesterone production after ovulation as a result of omega-3 PUFA supplementation pre- or post-ovulation. However there was no response to omega-3 PUFA on progesterone levels on d 10, 12 and 14 of gestation. Although there is some evidence for altered progesterone in cows fed omega PUFA from fish meal (Burke et al., 1997), others have found no effect (Mattos et al., 2002). In the present study, we measured peripheral, or systemic progesterone, from the jugular vein. Stefanczyk-Krzymowska et al. (1998) reported differences between systemic progesterone measured via the jugular vein and the local ovarian-utero blood supply. These authors concluded that implantation and placental elongation are influenced by elevated concentrations of steroid hormones supplied locally from the ovary rather than the systemic blood supply. Athorn et al. (2011) recently demonstrated that ovarian-derived progesterone measured in the local circulation from the vena cava was 3.5-fold higher and pulsatile in nature compared to peripheral progesterone concentration at d 10 of gestation when gilts were fed a higher feed allowance during pregnancy. Together, these findings suggest that nutritional changes can influence local progesterone level in the ovarian-utero circulation without altering peripheral progesterone. Therefore it is possible in the present study that omega-3 PUFA supplementation commencing after ovulation increased local ovarian progesterone around peri-implantation and therefore embryo survival, without altering peripheral progesterone.

In the present experiment, the results did not confirm that the increase in embryo survival was related to plasma EPA and DHA in sows around the time of implantation. A mechanism by which progesterone could be altered by dietary omega-3 PUFA is through the inhibition of ARA conversion to $PGF_{2\alpha}$ (Chartrand et al., 2003). However, there was no decline in plasma ARA when measured on d 10 and 14 of gestation in the present study. The fatty acid profile sampled on d 10 and 14 confirmed that the sows switched from the Omega-3 diet before mating to the Control diet during gestation resulted in a return of plasma EPA, and DHA to a lesser extent, to baseline levels by d 10. In the experiment described in Chapter 4, the increase in EPA and DHA in gilts occurred within three

days of being fed an omega-3 supplemented diet. The present study demonstrates the clearance of EPA and DHA from the circulatory system occurs within 10 d of cessation of dietary supplementation.

On the evidence of the results in Chapter 7 and the present study, it is proposed that two mechanisms are responsible for controlling embryo survival when diets supplemented with omega-3 PUFA. The first mechanism may be responsible for improved embryo survival due to omega-3 supplementation: an improved oocyte developmental competence when supplementation occurs prior to ovulation. A second mechanism may operate when supplementation occurs after mating such that an increase in local progesterone production and regulation of embryo survival via the ovarian-utero blood supply may improve embryo survival and litter size born. Furthermore, the overall response in embryo survival appears to have an upper limit suggesting that feeding omega-3 diets both before and after mating to alleviate constraints due to either of these mechanisms will maximize the response. In the following chapter, a possible mechanism where local progesterone production is related to specific prostaglandins derived from either omega-3 PUFA or a change in the omega-6:omega-3 ratio is examined.

In conclusion, the results from the present study showed that feeding omega-3 supplemented diets from fish oil to older sows either during lactation until mating, or after mating for the first four weeks of gestation improves embryo survival without affecting ovulation rate.

Chapter 9 Effect of addition of omega-3 derived PGE_3 and PGE_2:PGE_3 ratio on progesterone production from in-vitro granulosa cell cultures

9.1 Introduction

The synthesis and release of progesterone from the developing corpus luteum (CL) after ovulation is the principal mechanism governing early embryonic development (Geisert et al., 2005). During luteolysis, the thecal and granulosa cells of the follicle develop to form the CL (Smith et al., 1994; Murphy et al., 2001), undergoing a significant change in functionality from synthesizing oestrogens from thecal-derived androgens to synthesizing progesterone (Pescador et al., 1999).

In pigs, an increase in prostaglandin synthesis from the uterine endometrium during mid-oestrous causes the regression of the CL and the resumption of oestrus between d 18 – 21 (Geisert et al., 2005). Oestrogen released from the conceptus during trophoblast elongation acts as the signal for maternal recognition around d 11 – 13 post-mating, and stimulates progesterone synthesis from the CL during implantation (14 – 19 d) and throughout pregnancy (Waclawik et al., 2009). Prostaglandin E_2 (PGE_2) is synthesized from the endometrium and the conceptus, and appears to have a role in protecting the CL from the luteolytic action of $PGF_{2\alpha}$ (Wiesak et al., 1992; Li et al., 1993; Waclawik et al., 2009). In-vitro studies using luteal cell cultures from cyclic and pregnant sows with high levels of PGE_2 have demonstrated that PGE_2 increases progesterone production from luteal cells (Wiesak et al., 1992; Li et al., 1993; Armstrong et al., 2006). Uterine PGE_2 may act as an intermediatory around the time of ovulation enhancing the responses of follicle cells to the gonadotropin surge of LH thereby accelerating the chain of events that occur post-ovulation to form a functioning CL (O'Leary et al., 2006). PGE_2 is derived from the omega-6 ARA (ARA, C20:4) through the cyclooxygenase enzyme complex (COX-1 and COX-2; Caughey et al., 2005). Prostaglandin E_3 is derived from the omega-3 (EPA, C20:5) and as a series 3 prostaglandin, may be less bioactive than PGE_2 (Calder,

1996; Wathes et al., 2007). To our knowledge there are no published studies as to the effects of PGE_3 on progesterone production from porcine luteal cells.

The levels of EPA and ARA in plasma and tissues can be altered by the diet. Previously we found that supplementing diets with omega-3 fatty acids as fish oil improves litter size in sows (Chapters 3 and 7), and we have determined that this is due to a higher embryo survival with no effect on ovulation rate (Chapters 6 and 8). In Chapter 8, plasma progesterone was unaffected by changes in plasma omega-3 PUFA between 10 – 14 d of gestation. It is possible that the sampling of blood from the jugular vein as peripheral progesterone in Chapter 8 may not have reflected changes in progesterone directly from the ovary due to diet supplementation of omega-3 PUFA, as shown by others as a response to dietary energy (Athorn et al., 2011). A possible mechanism for the increased embryo survival in omega-3 supplemented sows observed in the previous chapter is that dietary EPA increases PGE_3 which stimulates progesterone synthesis from luteal cells in the local ovarian-uterine circulation. In this experiment, we examined the cellular progesterone response to prostaglandins derived from either omega-6 or omega-3 fatty acids, ARA and EPA, respectively. The aim of this experiment therefore was to determine if PGE_3 derived from omega-3 PUFA exerts a similar effect as PGE_2 to induce granulosa cell transition from a follicular function to a steroidogenic function producing luteal progesterone and consequently localized ovarian progesterone levels.

The present study tested the hypothesis that PGE_3 is equally effective as PGE_2 in stimulating progesterone synthesis from cultured granulosa cells, and that this effect is influenced by the ratio of PGE_2 to PGE_3.

9.2 Materials and Methods

To determine the effect of PGE and the relative importance of PGE_2 and PGE_3 on steroidogenesis, granulosa cells (GC) were cultured in-vitro with different concentrations of PGE_2 and PGE_3, in differing ratios of PGE_2:PGE_3, and in the presence or absence of hCG. Granulosa cells were cultured without prostaglandin (Control), or with 320 ng/ml PGE_2 or PGE_3, or a combination of PGE_2 and PGE_3 in ratios of 8:1, 4:1, 1:1 or 1:4 respectively. In a preliminary study, dose-response experiments were performed using different concentrations of PGE_2 and PGE_3 to determine the dose required to evoke maximal response in progesterone. Samples were replicated (n = 4) with addition of 50 µL of 0.10 IU/mL hCG to compare responses to PGE in the presence of hCG as a model of the pre-ovulatory LH surge. Cultures were incubated for 22 hours at 38^{o}C and the culture analyzed for progesterone and compared for treatment responses. The treatment design is presented in Table 9.1. The preparation of cultures was repeated in the following week from a second harvest of ovarian tissue.

9.2.1 Preparation of reagents

Solutions of PGE_2 and PGE_3 were prepared from stock solutions. Prostaglandin E_2 (Cayman Chemical Co., supplied by Cedarlane Laboratories, Ontario, Canada) was prepared to 1000 µg/mL by addition of ETOH and made up as 20 µL aliquots and stored at -20^{o}C until required. The stock was then diluted 1:100 with BTCM to 10,000 ng/mL. Prostaglandin E_3 (Cayman Chemical Co., supplied by Sapphire Bioscience, Waterloo, Australia) was prepared to 200 µg/mL by dissolving in methyl acetate and addition of ETOH in 20 µL aliquots (Stock PGE_3 A: 10,000 ng/mL) and stored at -20^{o}C until required. The PGE_3 stock was then diluted 1:20 with BTCM to 10,000 ng/mL. Solutions of 1000 µL of PGE_2 and PGE_3 were prepared by diluting stock solutions with BTCM (buffered tissue culture media) to create preparations with the following concentrations: 1600 ng/mL (Dilution B); 400 ng/mL (Dilution C); 100 ng/mL (Dilution D); and 25 ng/mL (Dilution E). Ratios of PGE_2:PGE_3 were

prepared using the Dilution B (1600 ng/mL) of PGE_2 and PGE_3 in 400 µL aliquots to create the following volumetric ratios: 8:1 (Dilution F), 4:1 (Dilution G), 1:1 (Dilution H) and 1:4 (Dilution I).

Stocks of hCG (LH) from 10 IU/mL dissolved in PBS + 0.1% BSA (phosphate buffered saline with bovine serum albumin) were prepared as 100 µL aliquots containing 10 mIU hCG/mL as stock and stored at -20°C. The stock was then diluted by adding 150 µL hCG stock to 2.85 mL BTCM 1:20 to give a diluted hCG solution of 0.5 IU/mL before adding to tissue cultures.

Stocks of IGF-1 were prepared at 10 µg/mL in PBS + BSA and stored at -20°C. Stock solution was diluted using 150 µL to 36 mL BTCM to a diluted IGF-1 solution of 41.4 ng/mL before adding to cell cultures.

9.2.2 Tissue collection and preparation

Reproductive tracts from 80 commercially slaughtered mixed parity sows (Large White x Landrace cross) were collected over two weeks from a commercial abattoir (Big River Pork Pty Ltd, Murray Bridge, SA) approximately 20 min after exsanguination. Sows had no dietary history of fish oil or any other sources of omega-3 PUFA supplementation. Sow ovaries were trimmed of tissue and placed in a vacuumflask at 37°C in a saline solution (0.9% NaCl) and transported to the laboratory and held at 37°C in a water bath. On each ovary, pre-ovulatory follicles between 3 – 8 mm diameter were aspirated using a 20 gauge 1 inch needle attached to a 10 mL test tube fitted with an airhose. The needle was rotated on the inside of the follicle to extract granulosa cells from the inner wall. Follicular fluid from several ovaries was pooled in aliquots of approximately 8 mL and placed in a heating block at 37°C whilst the remaining ovaries were processed. The period between abattoir collection and completed aspiration was approximately 90 min.

The follicular fluid from each aliquot was allowed to settle and cell contents were pipetted into a petri dish and assessed under a stereomicroscope with warming stage. Hepes buffered tissue culture

medium-199 (HTCM, ICN, Costa Mesa, CA, USA) was added to cover the bottom of the dish. Oocyte – cumulus complexes (OCC) and other debris were removed using a mouth pipette, leaving behind granulosa cells. Granulosa cells (GC) from each follicular aliquot were transferred to a 15 mL test tube (Falcon™, Franklin Lakes NJ, USA) and diluted with 5 mL HTCM. These were vigorously agitated and allowed to settle for 45 s. The settled pellet containing large sized GC were removed and the remaining mid-sized GC were agitated and allowed to settle under gravity for 30 min. The pellet of mid-sized GC was then removed and suspended with 5 mL bicarbonate tissue culture medium-199 (BTCM) + IGF1 (41.4 ng/mL) and centrifuged at low speed (500 rpm) for 5 min. The supernatant was removed by pipette and the cell pellet re-suspended in 5 mL BTCM+IGF1.

The concentration of GC in suspension was determined using a haemocytometer (Improved Neubauer, Webster Intl. England). 100 µL of the cell suspension was transferred to a 1.75 mL eppendorf™ tube and the cells dissociated by repeatedly pipetting the 100 µL volume. 15 µL of the dissociated cells were placed in a slide-well covered by a graduated cover slide. The concentration of cells in the sample was then determined. BTCM+IGF1 was added to the remaining 85 µL of cell suspension according to a dilution factor such that cell concentration was diluted to 0.833 x 10^6 cells/mL.

9.2.3 Tissue cultures

Tissue cultures were prepared in 1.75 mL eppendorf tubes. To each tube, 250 µL cultures were prepared in duplicate by adding 50 µL BTCM, 50 µL of either PGE_2 or PGE_3 and 150 µL of cell suspension in IGF1. These were then replicated in duplicate where BTCM was replaced by 50 µL hCG. The final concentration of each PGE sample (50 µL in 250 µL culture) was 5, 20, 80 and 320 ng/mL. Final cell concentration in culture was 0.5 x 10^6 cells/mL and final IGF1 concentration was 25 ng/mL. Final hCG concentration in the 250 µL culture was 0.5 IU hCG or 0.10 IU/mL. Cell

cultures were agitated then placed in racks in a humidified atmosphere incubator at 5% CO_2 in air at 38°C for 22 h. The time between slaughter and the start of incubation was approximately 4.5 h.

9.2.4 Progesterone assessment

The following day, cell cultures were removed from incubation and placed on crushed ice. Cultures were centrifuged for 4 min at 16,100 g. 50 µL of supernatant was extracted by pipette and stored in 1.75 mL eppendorf™ tube, labeled placed in liquid N and then frozen at -80°C for 48 h then stored at -20°C until analysis. Samples were analyzed for progesterone at the Obstetrics and Gynaecology Department, Medical School, The University of Adelaide, Adelaide, SA. Samples were prepared in duplicate by radioimmunoassay in 50 µL of a 1:10 dilution of plasma in duplicate according to the manufacturers instructions (IM1188; Beckman Coulter, Brea, CA, USA). The lowest detectable concentration was 0.5 pg/tube (equivalent of 1 ng/mL). The intra assay coefficient of variation was less than 10% and the inter assay coefficient of variation was less than 15% at 57 pg/tube (114 ng/mL).

9.2.5 Statistical analysis

Data was processed through a General Linear Model (GLM) analysis of variance for treatments in a randomized design model assuming equal variance (Sokal and Rohlf, 1981). The analysis of variance was performed using SPSS v18.0 (SPSS Inc., 1989-2009). Pairwise comparisons for each dependent variable between treatments were performed by Least Significance Difference tests and compared at $P < 0.05$. Probability values < 0.05 were described as significant, and $P < 0.10$ were used to describe trends.

9.3 Results

9.3.1 Main effect of hCG in mediating progesterone response to prostaglandin E

The effect of hCG addition was not significant (Table 9.2), nor was there a significant PGE x hCG interaction (P = 0.585), so data values of PGE with and without hCG addition were pooled. The preliminary dose response indicated that the maximum concentration of progesterone occurred at 320 ng/mL of both PGE_2 and PGE_3 (Figure 9.1). There was a significant increase (P = 0.017) in progesterone in culture media when either PGE_2 or PGE_3 were added at 320 ng/mL of culture media (Figure 9.2). Although the main effect of PGE was significant when compared to the control as BTCM (1.20 ± 0.36 ng/mL), there was no difference in progesterone production between PGE_2 (3.04 ± 0.59 ng/mL) and PGE_3 (2.55 ± 0.25 ng/mL; P = 0.413).

The effect of varying the ratio of PGE_2 to PGE_3 on progesterone production is summarized in Table 9.3. There was no significant increase in progesterone when PGE_3 was increased relative to PGE_2 (P = 0.602). Progesterone production in cultures with PGE_2:PGE_3 ratios between 8:1 and 1:1 were increased by 42% (P = 0.184). However the test for linearity between 8:1 and 1:1 ratios was not significant (P = 0.200). The main effect of hCG on progesterone production from varying PGE_2:PGE_3 ratios was also not significant (P = 0.201), nor was there a significant PGE x hCG interaction (P = 0.456). The effect of varying PGE ratio for pooled samples at 0 and 0.1 IU hCG is illustrated in Figure 9.3.

Table 9.1 Treatment plan of reagent addition to 150 µL granulosa cell culture for a final cell density of 0.5 x 10^6 cells/mL, 25 ng/mL of IGF-1 and 0.1 IU hCG/mL in final culture volume (250 µL)

Addition	PGE_3 response							
PGE_3, 50 µL[1]	0	0	PGE_3	PGE_3	0	0	PGE_3	PGE_3
hCG, 50 µL[1]	0	0	0	0	0.5	0.5	0.5	0.5
Sample ID	A1	A2	A3	A4	B1	B2	B3	B4
	PGE_2 response							
PGE_2, 50 µL[1]	0	0	PGE_2	PGE_2	0	0	PGE_2	PGE_2
hCG, 50 µL[1]	0	0	0	0	0.5	0.5	0.5	0.5
Sample ID	C1	C2	C3	C4	D1	D2	D3	D4
	PGE_2 : PGE_3 ratio response							
PGE_2 : PGE_3[1]	0	0	8:1	4:1	1:1	1:4	0	0	8:1	4:1	1:1	1:4
hCG, 50 µL[1]	0	0	0	0	0	0	0.5	0.5	0.5	0.5	0.5	0.5
Sample ID	E1	E2	E3	E4	E5	E6	F1	F2	F3	F4	F5	F6

[1]50 µL BTCM added where variable reagent was 0
Treatment plan was replicated twice over successive weeks.

Table 9.2 In-vitro progesterone production from cultured granulosa cells[1] derived from pre-ovulatory porcine ovarian follicles in the presence of PGE_2 and PGE_3 with and without hCG[2]

	Control (BTCM)		PGE_2 (320 ng/ml)		PGE_3 (320 ng/ml)		*P* value	
hCG (IU/mL)	0	0.1	0	0.1	0	0.1	PGE	hCG
Progesterone, (ng/mL)	1.62 ± 0.66	0.78 ± 0.23	3.78 ± 0.70	2.30 ± 0.88	2.68 ± 0.45	2.42 ± 0.29	0.015	0.088

[1]GC concentration in tissue culture media 0.5 x 10^6 cells/mL.
[2]hCG concentration in tissue culture media 0.1 IU/mL
Mean ± SE values are the sum of 4 replicates

Table 9.3 Treatment progesterone response from cultured granulosa cells[1] in the presence of PGE_2 and PGE_3 in different ratios with and without hCG[2]

	PGE_2 : PGE_3 ratio[3]									
	8:1		4:1		1:1		1:4		*P* value	
hCG (IU/mL)	0	0.1	0	0.1	0	0.1	0	0.1	Ratio	hCG
Progesterone (ng/mL)	2.75 ± 0.65	2.05 ± 0.65	2.55 ± 0.63	3.32 ± 0.87	2.85 ± 0.71	3.9 2 ± 1.10	2.12 ± 0.52	3.65 ± 0.35	0.602	0.201

[1]GC concentration in tissue culture media 0.5 x 10^6 cells/mL.
[2]hCG concentration in tissue culture media 0.1 IU/mL.
[3]PGE_2 and PGE_3 concentration at 320 ng/mL
mean ± SE values are the sum of 4 replicates.

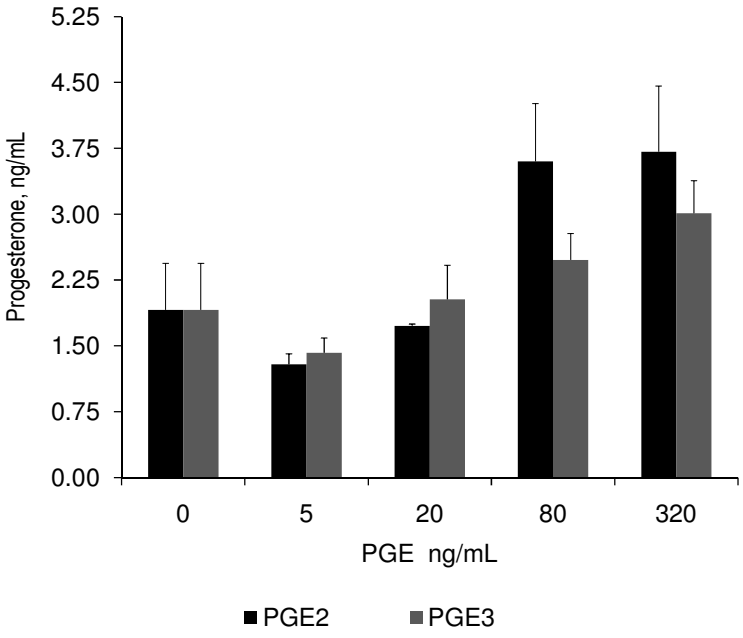

Figure 9.1 Raw mean ± SE for a dose-response of progesterone in a preliminary sample (n = 4 per treatment) to determine the experimental dose for PGE

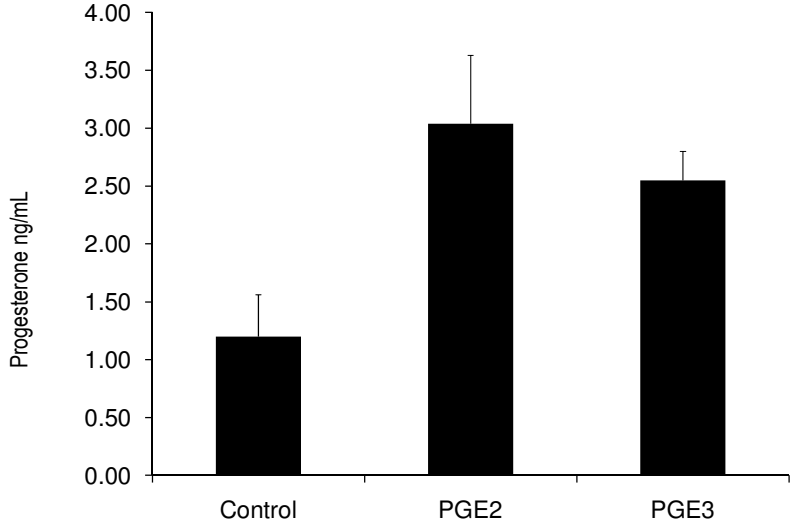

Figure 9.2 Effect of PGE_2 and PGE_3 at 320 ng/mL ($P < 0.05$) compared to Control (BTCM) on in-vitro progesterone production from granulosa cells (0.5×10^6/mL) in tissue culture media (n = 8 per treatment). Data pooled between samples cultured in presence or absence of hCG.

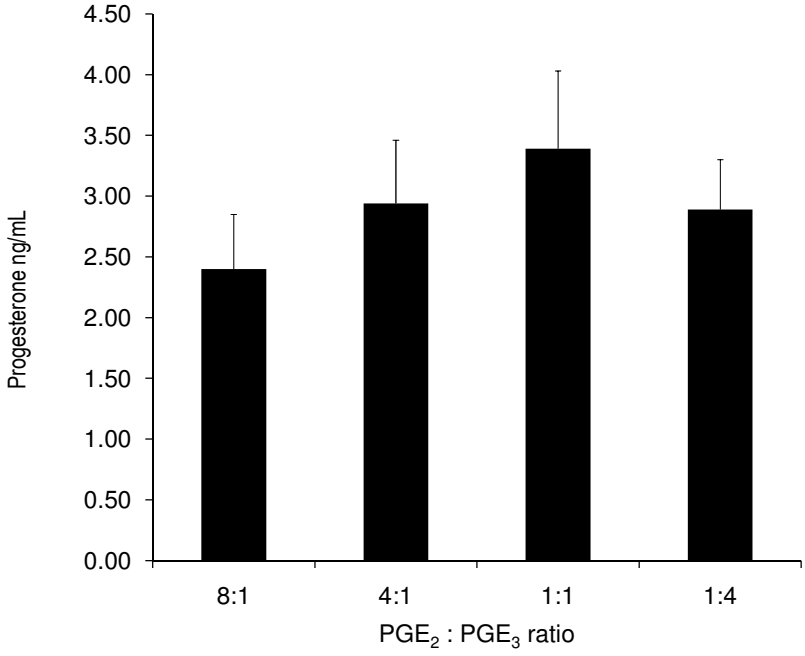

Figure 9.3 Effect of PGE_2 : PGE_3 ratio using solutions of PGE at 320 ng/mL concentration on progesterone production from granulosa cells (0.5 x 10^6/mL) in tissue culture media (n = 8 per treatment). Data pooled between samples cultured in presence or absence of hCG.

9.4 Discussion

The present study was undertaken to determine the bioactivity of the omega-3 derived PGE_3 in stimulating progesterone production from granulosa cells. The results from this study showed progesterone production was significantly increased in the presence of high levels of PGE_3, as well as supporting previous studies by others who demonstrated a stimulatory effect of PGE_2 on progesterone production from porcine granulosa cells recovered from pre-ovulatory follicles (Wiesak et al., 1992; Li et al., 1993; Armstrong et al., 2006). This is the first time to our knowledge that a steroidogenic response to the omega-3 derived PGE_3 has been investigated.

The prostaglandin, PGE_2, has a role in regulating luteolysis and the maintenance of the CL and the conceptus during early pregnancy (Smith et al., 1994). Gene expression of COX-2 in the uterine epithelium increases 80-fold during peri-implantation (Geisert et al., 2006), with a resulting increase in PGE_2 in pregnant gilts compared to non-pregnant gilts and a concomitant increase in $PGF_{2\alpha}$ in the uterine fluid (Geisert et al., 1982). Uterine infusion of PGE_2 has also been shown to increase plasma progesterone (Akinlosotu et al., 1986; Stefanczyk-Krzymowska et al., 2006). In the pig, luteolytic $PGF_{2\alpha}$ is redirected from the ovarian-uterine vascular system to the uterine lumen in order to maintain pregnancy (Senger, 1997). There are a number of roles of PGE_2 which include acting as an initiating agent for follicle transformation to the CL after ovulation and acting as an inhibitor of the luteolytic $PGF_{2\alpha}$ during early pregnancy (Smith et al., 1994). Because the endometrium and trophoblast synthesize PGE_2 during implantation as a luteoprotective mechanism (Waclawik et al., 2009), changes in dietary supply of the favoured precursor, ARA, could determine pregnancy outcomes. Chartrand et al. (2003) studied the effect of changes to the source of dietary fat fed to gilts on prostaglandin synthesis. Comparing linseed oil (high ALA) with tallow, they were able to change the plasma PUFA profile, reducing ARA and increasing EPA. The production of $PGF_{2\alpha}$, and to a lesser extent PGE_2, was reduced in the gilts fed linseed oil diets compared to gilts fed diets with tallow and they concluded that omega-3 ALA inhibited the supply of ARA, the precursor to $PGF_{2\alpha}$

and PGE_2. There are no other published studies in pigs where long-chain omega-3 EPA and DHA supply have been related to PGE_2 or PGE_3 and the associated effects on luteal development and steroidogenesis. In cattle, Hinckley et al. (1996) reported a dose-dependent reduction in progesterone when EPA was added directly to cultured bovine luteal cells at high levels (> 100 ng/mL). However, they did not measure the resultant PGE_3 or PGE_2 levels to permit a comparison with the levels used in this present study. The 3-series prostaglandins are regarded as less biologically active than the 2-series family (Caughey et al., 2005; Wathes et al., 2007) and up until now, the effectiveness of PGE_3 in eliciting a progesterone response has not been reported.

The production of progesterone by PGE_3 was similar to that produced by PGE_2 in-vitro. Choosing the level of PGE concentration at 320 ng/mL is supported by data from Wiesak et al (1992) who reported that luteal cells cultured in-vitro with PGE_2 at 100 and 1000 ng/mL produced the largest progesterone response. In contrast to the findings of Hinckley et al. (1996), the preliminary dose-response data in the present study did not indicate a negative effect of PGE_3 on progesterone, but rather an increasing progesterone response was observed.

Including hCG as an analogue for LH had no effect on progesterone production in the presence of PGE_2 or PGE_3. This result differs to that achieved by Armstrong et al. (2006) who reported a five-fold increase in progesterone and LHRH receptor activity when hCG was added to PGE_2. Li et al (1993) also reported that LH at 20 ng/mL induced 4 to 5-fold increases in progesterone from incubated cell cultures. The results from the addition of hCG to decreasing PGE_2:PGE_3 ratios in the current experiment suggest an enhanced progesterone response, however the magnitude was less than expected with only 1 to 1.5-fold increase in progesterone due to hCG addition. This may have been due to insufficient hCG used in this experiment.

In human nutrition, it has been suggested that there is an optimal balance or ratio of dietary omega 6:omega-3 fatty acids for health and development (Simopoulos, 2005). The concept of an optimum

concentration of dietary omega-3 fatty acids has also been proposed by Rooke et al. (2001d) in pregnant sows in order to maintain ARA at suitable levels whilst increasing EPA and DHA for foetal development and post-natal growth. There was a non-significant trend towards a linear increase in progesterone production by increasing the addition of PGE_3 to PGE_2 up to a combination ratio of 1:1. This provides support for the hypothesis that PGE_3 could enhance the steroidogenic response from luteal tissue in the presence of PGE_2. Increasing PGE_3 might also produce a similarly synergistic response as PGE_2 in lowering $PGF_{2\alpha}$ from the ovarian and uterine blood supply, which warrants further investigation.

In conclusion, the present study has shown for the first time that both PGE_2 and PGE_3 have a steroidogenic response on the luteal cells from the pre-ovulatory follicle, stimulating progesterone production. There also appears to be a synergistic response in progesterone production when the PGE_2:PGE_3 ratio is reduced by adding PGE_3 to cell culture media. It is proposed that reducing the PGE_2:PGE_3 ratio through supplementation of omega-3 PUFA as fish oil could enhance local ovarian progesterone levels which in turn influences embryo survival.

Chapter 10 General Discussion

Dietary fats are essential for the supply of fatty acids as a component of cell membranes and as substrates for eicosanoids in animal diets (Smith et al., 1983). Although there is a growing body of evidence supporting findings that fertility in livestock is affected by dietary PUFA independent of energy, the mechanism by which omega-3 PUFA influences reproduction are largely unknown (Wathes et al., 2007). Similarly, there is a paucity of data published on the effect on reproduction performance in breeding gilts and sows when fed isocaloric diets supplemented with omega-3 fatty acids. The objective of this thesis was therefore to investigate the reproductive response when long-chain omega-3 PUFA from fish oil were added to diets fed to gilts and multiparous sows. Secondly, the experiments evaluated possible mechanisms that could be responsible for these responses. The findings from this thesis have shown that litter size born is improved in sows fed diets supplemented with low inclusion levels of fish oil. This response was not observed in gilts but was consistently demonstrated in higher parity sows and occurred without an increase in energy intake. The increase in litter size in supplemented sows appeared to be due to an increase in early embryonic survival as ovulation rate was unaffected. Other reproductive parameters including pregnancy and farrowing rate; sow feed intake and body weight and fat loss in lactation; piglet preweaning growth performance; sow and piglet mortality; resumption of oestrus and weaning to oestrus interval were unaffected by supplementation.

Sow productivity is highly dependent on fertility and prolificacy over several litters. Low litter size and reproductive failure are the two largest contributors of early sow culling limiting longevity and lifetime productivity in the sow herd (Hughes and Varley, 2003). One of the factors influencing the productivity of commercial herds in recent years is a decline in litter size as sows become older (Hughes and Varley, 2003; Levis, 2005). Comparatively, older sows now have litters with fewer piglets than was previously observed from the same parities. As a result, older sows with low litter

sizes are more likely to be culled as being un-productive thereby contributing to a lower profitability of piggeries (Levis, 2005). High sow turnover is also a concern amongst consumers and sections of the community who demand sustainable livestock production on ethical grounds. The results from the present studies are therefore important to the pig industry as it strives to be highly productive and profitable, operating in a manner which is acceptable by the community.

There are several studies published in other species that report an improvement in reproductive performance due to omega-3 fatty acids (reviewed by Wathes et al., 2007). However, studies in pigs are limited. Palmer (1970) reported an increase in farrowing rate and litter size born to sows of parity 1 and parity 2 when whole fishmeal in the diet was fed to gilts before mating and then continued over two consecutive reproductive cycles. However, their results were criticized as the dietary supply of other nutrients, including selenium and vitamin E, was imbalanced (Baker et al., 1974). Feeding whole fishmeal may also have changed the dietary intake of energy as Palmer et al. (1970) observed an increase in gestation weight gain. As such, the diets in the present studies were formulated to contain equal amounts of energy and protein, and used the same vitamin and mineral premix. Fish oil, rather than fish meal was used as a source of long-chain omega-3 PUFA, EPA and DHA, which partially replaced tallow in order to keep energy and crude fat concentration constant between the treatments. The analyses of dietary fatty acids confirmed the experimental diets essentially differed only in long-chain omega-3 fatty acid supply of EPA and DHA, with omega-6 PUFA and all other nutrients kept constant.

In the first study in Chapter 3, we found that sows fed a lactation diet supplemented with long-chain omega-3 PUFA from fish oil had an increased subsequent litter size born (total and live born) by 1 piglet/litter compared with sows fed an unsupplemented diet during prefarrowing and lactation. This study highlighted the potential for dietary supplementation of omega-3 fatty acids as a nutritional approach to improve sow reproductive performance. This is the first full report of an increase in sow

reproductive performance due to an increase in the supply of long-chain omega-3 PUFA as fish oil to sows fed prefarrowing through to weaning. In an abstract, Webel et al. (2004) reported that sows fed a supplement containing omega-3 PUFA increased subsequent litter size born in sows fed prefarrowing during lactation, and continued supplementation to mating. However, other published studies have failed to demonstrate any response on litter size to sows fed omega-3 supplemented diets from fish oil (Perez-Rigau et al., 1995; Rooke et al., 2001b; Rooke et al., 2001c; Mateo et al., 2009).

Although they reported a lack of response in weaned sows fed supplemented diets with omega-3 PUFA, Perez-Rigau et al. (1995) reported a tendency for embryo survival to increase in gilts fed a diet containing fish oil compared to corn starch, coconut oil and soybean oil 35 d before mating until slaughter at six weeks of gestation. In Chapters 4 and 5, experiments were designed to investigate the effect of the duration and level of omega-3 supplementation on reproductive performance in gilts. There was no increase in litter size born to gilts supplemented for either three or six weeks prior to mating. Other reproductive parameters including the onset of oestrus; proportion of gilts mated; and farrowing rate were similar between dietary treatments. In the industry, a long duration of feeding supplemented diets prior to mating has been recommended, although there is little published data to support this. In their abstract, Webel et al. (2004) recommended that a feeding period of 35 d or more prior to mating was required to maximize the response to a dietary supplement of long-chain omega-3 PUFA. Similarly, Spencer et al. (2004; abstract) fed supplemented diets in their gilt study for a minimum of 30 d and reported an increased litter size using the same supplement as Webel et al (2004). In the experiments described in Chapter 4, there was no difference in litter size or farrowing rate between gilts fed Omega-3 for 3 weeks (average duration of supplementation of 24 d) compared to gilts fed Omega-3 for 6 weeks (average duration of 47 d) prior to mating. In these studies, it is reported that plasma EPA and DHA increased within 3 d after the commencement of

supplementation, suggesting that animals do not need to accumulate stores of omega-3 PUFA in fat depots or tissues for plasma PUFA levels to be elevated.

In Chapter 4, an increase in age at mating was recorded in gilts fed Omega-3 for 6 weeks prior to mating compared to unsupplemented gilts and those gilts fed Omega-3 for 3 weeks. Although mating age was delayed, supplementation did not affect the incidence of anoestrus or delay the onset of puberty. In Chapter 5, there was no evidence that the onset of oestrus was adversely affected when gilts were fed diets containing fish oil. In subsequent experiments in sows, there was no evidence of a longer weaning to oestrus interval or anoestrus. Therefore, it seems unlikely that omega-3 PUFA influences the hypothalamic-pituitary-ovarian axis and gonadotropin hormone levels in relation to these parameters.

In Chapter 5, supplementation was changed from Chapter 4 so that diets were fed to gilts from 24 weeks of age and beyond mating through to early gestation to replicate the feeding program of Perez-Rigau (1995). Omega-3 diets with 3 or 6 g fish oil/kg diet were evaluated with an unsupplemented diet. There was no effect of supplementation on pregnancy rate or ovulation rate in gilts, although there was a trend for an increase in embryo survival in supplemented gilts that was maximal at 3 g fish oil/kg diet (Chapter 5). Taken together, the findings from the two gilt studies showed that the duration of supplementation did not affect reproductive performance, and that increasing the amount of omega-3 PUFA by adding more fish oil above 3 g/kg did not increase embryo survival. Although the level of EPA and DHA in plasma was substantially increased, only a small, non-significant increase in litter size and embryo survival was observed in these gilt studies, suggesting that the high inherent embryo survival of gilts (82%) may preclude a significant response to supplementation. These results support the findings of Estienne et al. (2006) who concluded that ovulation rate and embryo survival was unaffected by feeding a diet supplemented with EPA and DHA omega-3 PUFA to gilts with a high level of fertility. When compared to the litter size born and

farrowing rate responses from sows in Chapter 3, it was proposed that older parity sows may be more responsive to omega-3 supplementation from fish oil. Foxcroft et al. (2006) reported from sow slaughter studies that although ovulation rates were largely constant in young and old parity sows, embryo survival declined with parity. It was therefore decided to continue the investigations on reproductive response to omega-3 supplementation in older sows.

In Chapter 6, mature sows (parity 5 and older) were used to confirm the effect of omega-3 supplementation on litter size observed previously in Chapter 3, and to determine whether the effect was due to improved embryo survival. Sows were fed a supplemented diet of 3 g fish/kg from prefarrowing continuing to mating before being placed on an unsupplemented gestation diet until slaughter at 23 d after mating. Embryo survival and embryo number at 23 d of gestation was increased in supplemented sows compared to controls. This study confirmed the finding in sows from Chapter 3 where the number of piglets born was increased subsequent to being fed a diet supplemented with omega-3 PUFA as 3 g fish oil/kg prior to mating. Two other findings were also important from this study. Firstly, embryo survival rather than ovulation rate was identified as the underlying cause for the increase in litter size, and secondly, there was no effect of diet on sow intake; weight loss or fatness loss during lactation; or piglet weight gain, confirming the conclusion in Chapter 3 that the reproductive response to omega-3 supplementation is due to the change in fatty acid profile rather than an increase in energy intake.

From Chapter 6, it was concluded that reproductive performance as litter size is enhanced in older sows fed omega-3 supplemented diets from 3 g fish oil/kg of diet prior to mating as a result of an increase in embryo survival. Post-mating nutrition and feeding level, especially high energy intake, has been shown to reduce embryo survival, possibly through progesterone clearance (Dyck and Strain, 1983; Jindal et al., 1997). Pre-mating nutrition has also been shown to affect ovulation rate (Grandhi, 1988) and oocyte quality when energy intake during lactation is altered (Zak et al., 1997a;

Zak et al., 1997b; Ashworth and Antipatis, 1999). Effects of specific fatty acids or fat source on oocyte quality have not been widely reported in pigs, though an increase in oocyte quality in sheep fed isocaloric diets supplemented with rumen-protected calcium soaps of omega-3 PUFA fish oil was reported by Zeron et al (2002). Because sow intake and live weight change in lactation was unaffected, it was concluded the increase in embryo survival when sows were fed a diet high in omega-3 PUFA up to the time of mating occurred independent of an effect of energy on oocyte growth and follicular development. In a parallel experiment, ovaries were collected 4 d after weaning from unmated sows (parity 5 and older) fed omega-3 diets prefarrowing, during lactation and postweaning to assess oocyte development (Mitchell et al., 2010). Oocytes were collected from pre-ovulatory follicles, matured and fertilized in-vitro. Fertilized oocytes from sows fed omega-3 diets containing 3 g fish oil/kg had a higher development rate to the blastocyst stage (d 6 post-fertilisation) compared to sows fed an unsupplemented diet (Mitchell et al., 2010). From these results, it was proposed that the embryo survival observed in-vivo in sows fed supplemented diets prior to mating in Chapter 6 is due to improved oocyte quality. However the effect of omega-3 PUFA supplementation after mating on the uterine environment and the steroidogenic capacity of corpora lutea (CL) to produce progesterone had not been examined. Therefore it was proposed in Chapter 7 and 8 to evaluate the response to supplementation either for four weeks before mating or four weeks after mating, and to test the hypothesis that these responses were additive when feeding omega-3 supplemented diets continuously from prefarrowing through early gestation.

The main finding in Chapter 7 was there were additive effects on subsequent piglets born when sows were fed omega-3 diets both before and after mating. Weaning to oestrus interval and farrowing rate were unaffected by dietary treatment. Subsequent litter size was 15% higher than unsupplemented sows at parity 4 – 7 when fed during lactation and continued for four weeks of gestation. The response to subsequent litter size born in sows commencing supplementation from mating was also higher than the unsupplemented sows (an increase of 11% total born). Although subsequent litter

size born live was less responsive than total litter size born (Chapter 7), the trends followed those for total born. Using the same diets and feeding regimen in Chapter 8, the supplementation regimen of Omega-3 – Omega-3 in sows resulted in a higher embryo survival compared to unsupplemented sows, with ovulation rate unaffected. In addition, the results in Chapter 8 are the first report of an increase in embryo survival when sows are fed omega-3 diets supplemented as 3 g fish oil/kg commencing from mating and continuing through early gestation. Rooke et al. (2001c) investigated the effect of omega-3 supplementation from fish oil commencing 3 d after mating and throughout gestation on farrowing and neonatal viability in older parity sows, and reported that litter size was unaffected by gestation feeding of omega-3 PUFA . In their study, Mateo et al. (2009) fed pregnant gilts an omega-3 PUFA supplement from d 60 of gestation until weaning and then commenced supplementation again on d 60 in the second parity. They also observed no difference between supplemented and unsupplemented sows on either first or second parity litter size born.

The magnitude of the response in subsequent total born recorded in Chapter 7 in older sows supports the hypothesis there are additive effects of feeding omega-3 supplemented diets before and after mating and continuing through early gestation. However, the mechanism responsible for the improvement in embryo survival remains unclear. Enhanced oocyte quality may have contributed towards a higher embryo survival on Omega-3 – Control sows but it does not explain the similar level of embryo survival in the Control – Omega-3 sows which commenced supplementation after ovulation (Chapter 8). The conceptus and uterine endometrium uptake of omega-3 PUFA may have been improved with supplementation in early gestation in Control – Omega-3 sows and enhanced embryo development and survival, as proposed by Brazle et al. (2009). In Chapter 8, there was no difference in embryo survival between Omega-3 – Control and Control – Omega-3 sows by 23 d of gestation. However, the result in Chapter 7 where a higher number of sows was used suggests a greater response in litter size born in Control – Omega-3 sows (11% higher than Control – Control group) compared to the Omega-3 – Control group (5% higher than Control –

Control group). A possible explanation for this difference between embryo survival at 23 d (Chapter 8) and subsequent litter size born (Chapter 7) is that omega-3 diets fed during early gestation contributes toward a more receptive uterus and a better environment for foetal survival as the pregnancy advanced.

By feeding diets with low levels of fish oil during early gestation, plasma EPA and DHA were substantially increased without affecting ARA levels by d 10 of gestation (Chapter 8). As a luteolytic agent, $PGF_{2\alpha}$ causes regression of the CL and directly inhibits the enzymes involved in the production of luteal progesterone from cholesterol (Smith et al., 1994; Wathes et al., 2007). Smits et al. (2007) reported that increasing dietary EPA and DHA from fish oil reduced plasma levels of omega-6 ARA in pigs, the precursor for $PGF_{2\alpha}$, and this was confirmed again in gilts in Chapter 5. A possible mechanism proposed in Chapter 8 was that feeding a diet high in omega-3 PUFA would decrease $PGF_{2\alpha}$ by reducing ARA concentration, resulting in the maintenance of the CL and higher circulating progesterone levels. Chartrand et al. (2003) fed gilts a diet high in linseed oil, a source of omega-3 ALA and reported a decrease in $PGF_{2\alpha}$ concomitant with decreased ARA, when plasma ALA and EPA were elevated. Therefore, in Chapter 8 it was proposed that increased levels of dietary omega-3 PUFA would inhibit plasma ARA, the primary substrate for $PGF_{2\alpha}$, and thus improve embryo survival via elevated progesterone levels. However, plasma ARA was not reduced in the presence of high EPA and DHA at d 10 – 14. As progesterone was not related to differences in plasma EPA and DHA, it is likely, though not confirmed, that increased EPA and DHA did not decrease $PGF_{2\alpha}$ during peri-implantation. Another possible explanation is that supplementation may have affected progesterone in the local ovarian-utero circulation, but not peripheral progesterone concentration which was unaltered by supplementation (Chapter 8). Athorn et al (2011) recently reported that gilts sampled from the vena cava had a different pattern of progesterone concentration in plasma than progesterone measured from blood samples taken from the jugular vein in response to changes in feeding level. Therefore, peripheral progesterone as we measured may not be as

sensitive a response to omega-3 PUFA as circulating progesterone concentration at the ovary-utero level. Subtle changes in ovarian progesterone due to dietary changes in omega-3 PUFA may not have been detected as a consequence of the sampling procedure used in these experiments. Others have reported that embryo survival and implantation are regulated by steroid hormones derived from this local circulatory system (Stefanczyk-Krzymowska et al., 1998; Stefanczyk-Krzymowska et al., 2006).

The role of prostaglandins in the regulation of ovulation, luteinisation and embryo implantation when altered by changes in omega PUFA from the diet remains unclear. After ovulation, prostaglandin PGE_2 is involved in transformation of the follicle cells into granulosa cells of the CL, as well as overcoming the luteolytic action of $PGF_{2\alpha}$ (Smith et al., 1994). Around the time of implantation, there is a substantial increase in the endometrium of PGE_2 and an up-regulation of COX-2, the enzyme responsible for prostaglandin synthesis (Geisert et al., 1982; Geisert et al., 2006). Some researchers have reported an inhibitory effect of EPA and DHA on PGE_2 in ovine cell cultures from CL tissue, with a decrease in progesterone, and a concomitant increase in $PGF_{2\alpha}$ (Hinckley et al., 1996). In Chapter 9, we investigated the progesterone production by granulosa cells in-vitro in response to the addition of PGE_3, which is formed from EPA rather than ARA in the COX-1 and COX-2 eicosanoid metabolism. Although it is well established in the literature that PGE_2 increases progesterone production and enhances steroidogenesis from luteal cells (Wiesak et al., 1992; Li et al., 1993), the results from Chapter 9 are the first report showing that PGE_3 also stimulates progesterone production from granulosa cell cultures, and that there was evidence for a synergistic response when added in conjunction with PGE_2 from 8:1 to 1:1 as a ratio of PGE_2 : PGE_3.

Thus, the results of Chapter 9 suggest that increasing the supply of EPA, and consequently PGE_3, may improve steroidogenesis and progesterone production by granulosa cells which in turn influences embryo development. However, the mechanism for improved embryo survival may also

be dependent on a dietary level of omega-3 PUFA that does not suppress ARA and hence PGE_2. Although peripheral plasma progesterone levels at peri-implantation were similar between unsupplemented sows and those fed omega-3 diets either before mating, after mating or both (Chapter 8), a response on PGE_3 or localised ovarian progesterone synthesis in gestation may be a plausible mechanism by which embryo survival is improved. It is possible that other non-steroidogenic effects may also be involved in the dietary response on embryo survival around the time of implantation due to synergistic actions of PGE_3 with PGE_2.

Embryo survival recorded in Chapter 8 was similar between sows supplemented either before ovulation (Omega-3 – Control) or after ovulation (Control – Omega 3). This suggests that two mechanisms may be acting to increase embryo survival in response to omega-3 PUFA supplementation. One mechanism, as suggested by our preliminary study which improves oocyte developmental competence when sows are supplemented prior to ovulation (Mitchell et al., 2010), would explain the increase in embryo survival observed in Chapter 6 and 8 and high litter size born in Chapter 3 and 7 in older sows supplemented during lactation and up to mating compared to controls. The second mechanism proposed is an increase in the local ovarian-uterine progesterone level due to either an increase in omega-3 derived PGE_3 or through a reduction in PGE_2:PGE_3 ratio in response to omega-3 supplementation during early gestation. In Chapter 9, PGE_3 in combination with PGE_2 increased progesterone synthesis in-vitro. The proposed mechanism for enhanced steroidogenesis would improve embryo survival due to luteal support through local ovarian-uterine circulation in sows that did not commence supplementation until after ovulation and in the absence of a dietary effect on occyte quality. Although it was hypothesized there may be additive effects on embryo survival when these two mechanisms operate concurrently, this was not supported by the data in Chapter 8, suggesting there is an upper limit to embryo survival in older sows. Hence, feeding omega-3 PUFA at one or other time is likely to be as effective as feeding supplemented diets across both periods.

In summary, these findings supported the hypothesis that feeding isocaloric diets with omega-3 PUFA as EPA and DHA from the addition of low levels of fish oil improves reproductive performance in sows. The magnitude of this response is dependent on parity and is greatest in older sows that have a propensity for low embryo survival. Supplementation with omega-3 PUFA as 3 g fish oil/kg of diet did not affect ovulation rate in either gilts or sows, but consistently improved embryo survival and subsequent number of piglets born in older parities by an average of 9% across experiments. Increasing the level of specific long-chain omega-3 fatty acids from fish oil improves embryo survival, although the mechanism(s) still need to be confirmed. Peripheral progesterone levels were not associated with increases in plasma EPA and DHA around peri-implantation. Conversely, plasma ARA was not consistently decreased by dietary supplementation with omega-3 PUFA using 3 g fish oil/kg. It is proposed that two mechanisms are involved in increasing embryo survival: 1) an enhanced oocyte developmental competence when supplementation occurs during lactation and prior to ovulation; and 2) an increase in local progesterone synthesis due an increase in the omega-3 PGE_3 when supplementation occurs during early pregnancy. .

In conclusion, litter size is increased when sows are fed diets supplemented with long-chain omega-3 PUFA supplied from fish oil. In particular, older parity sows are more responsive than gilts and young sows. This response is mediated through an increase in embryo survival with the mechanisms governing this response appearing to operate on oocyte quality and/or luteal progesterone synthesis. Future work should further explore these mechanisms in older sows and confirm the effect on embryo survival in response to omega-3 fatty acids.

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