“We still do not know one thousandth of one percent of what nature has revealed to us.”
- Albert Einstein (1879 – 1955)

University of Alberta

Litter birth weight phenotype and maternal n-3 long-chain polyunsaturated fatty acid supplementation in pigs

by

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Abstract

Research reported in this thesis investigated effects of n-3 polyunsaturated fatty acid (LCPUFA) supplementation, litter birth weight phenotype, and their possible interactions, on reproductive performance of the gilt and sow and postnatal performance of the litter. In an initial study, LCPUFA supplementation to gilts from day 60 of gestation improved litter growth until the end of the nursery period, increased pre-weaning mortality, but did not affect subsequent reproductive performance of the dam. Consistent with the hypothesis that changes to the component traits affecting litter size (ovulation rate and embryonic survival) lead to intrauterine crowding (IUC) of embryos and intra-uterine growth restriction (IUGR) in a proportion of higher parity sows, data from an initial collaborative study confirmed that, compared to medium (MBW) or high (HBW) birth weight litters, low birth weight (LBW) litters had lighter placentae at term and stillborn pigs born showed benchmarks of IUGR such as a higher brain:liver weight ratio. LBW litters also had higher pre-weaning mortality and lower growth rates throughout the growth period and needed 9 more days to reach a fixed market weight than HBW litters. Carcass quality was similar between litter birth weight phenotypes. As litter birth weight phenotype was found to be repeatable within sows, and given the results from the initial gilt study, a second sow study was performed to investigate interactions between litter birth weight phenotype and LCPUFA supplementation to sows during the rebreeding period, gestation and lactation. Compared to untreated control sows, LCPUFA supplementation reduced litter size at birth and increased postnatal growth of medium/high birth weight (MHBW) but not LBW litters. After weaning, body weight was only improved by LCPUFA supplementation when no competition for food or space occurred, and had no effect on ADG, ADFI or feed efficiency. Carcass fat depth was

higher and lean meat percentage lower, when sows were supplemented with LCPUFA. Overall, therefore, the economic benefits of LCPUFA supplementation are questionable. However, the swine industry should strive to find ways to decrease the number of LBW litters: Until this has been achieved, management strategies to deal with LBW litters are critical.

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Figure 6-4. Body weight change during the grow-finish period in barn B littermates from sows fed diets either with (mLCPUFA; n=24 pens) or without (CON; n=24 pens) enrichment with marine-oil based n-3 LCPUFAs. Overall effect of treatment, P=0.57: effect of time, P < 0.001: interaction between treatment and time, P = 0.98.
Data are the LSMeans + Standard Error 186

Figure 7-1. Relationship between litter size and litter average birth weight for litters from the trial described in Chapter 5, divided into litters that had piglets with individual birth weight in four different quartiles (0.80-1.25 kg, 1.25-1.50 kg, 1.50-1.75 kg and 1.75-2.50 kg; All quartiles, n=68) and litters that did not have piglets with individual birth weight in all four quartiles (Not all quartiles, n=66). Only piglets that were alive at the time of measuring birth weight were taken into account in this analysis. The litters between the two vertical lines are the litters with 9 to 16 total pigs born, which were classified as low, medium or high birth weight (see Chapter 5) 215

List of Abbreviations

AA	arachidonic acid
ADFI	average daily feed intake
ADG	average daily gain
AI	artificial insemination
ALA	α-linolenic acid
ATP	adenosine triphosphate
ave	average
BA	born alive
BdW	body weight
BSA	bovine serum albumin
bw / BW	birth weight
°C	degrees Celsius
CL	Corpus Luteum
CON	control
CORR	correlation
COX	cyclooxygenase
CV	coefficient of variation
d	day
DGLA	dihomo-γ-linolenic acid
DHA	docosahexaenoic acid
DNA	Deoxyribonucleic acid
DPA	docosapentaenoic acid
ELISA	enzyme-linked immunosorbent assay
EPA	eicosapentaenoic acid
g	gram
g	gravitational force
GLA	γ-linolenic acid
GLM	general linear model
GSI	gonadossomatic index
h	hour
HBW	high birth weight litter
HW	high individual birth weight

IGF	insulin-like growth factor
IgG	immunoglobulin G
intest.	small intestine
IU	international units
IUC	intrauterine crowding
IUGR	intrauterine growth restriction/retardation
kg	kilogram
KIU	kilo international units
l	litre
LA	linoleic acid
LBW	low birth weight litter
LCPUFA	long-chain polyunsaturated fatty acid (20 or more C atoms)
LH	luteinizing hormone
lit	litter
LOX	lipoxygenase
LSMeans	least square means
LTB, C, E	leukotrienes B, C or E
LW	low individual birth weight
LXR	liver X receptor
M	molar
m^2	square metre
mm	millimeter
MBW	medium birth weight litter
ME	metabolizable energy
mg	milligram
MHBW	medium/high birth weight litter
min	minute
MJ	Mega Joule
ml	millilitre
MUFA	mono-unsaturated fatty acid
n	number
ng	nanogram
NO	nitric oxide
n-3	omega-3

n-6	omega-6
PBMC	peripheral blood mononuclear cell
PGE	prostaglandin E
PGF	prostaglandin F
PPAR	peroxisome proliferator receptor
PUFA	polyunsaturated fatty acid
R&D	Research and Development
RSD	residual standard deviation
SAS	Statistical Analysis Software
SB	stillborn
S.E.D.	standard error of the difference
S.E.M.	standard error of the mean
SFA	saturated fatty acid
SGA	small for gestational age
SNP	single-nucleotide polymorphism
SRDP	Swine Reproduction and Development Group
SREBP	sterol regulatory element-binding protein
SRTC	Swine Reproduction and Technology Centre
St.Dev.	standard deviation
TB	total born
TBS	Tris-buffered saline
TID	true ileal digestible
Trt	treatment
TW	testicular weight
TXA	thromboxane
UHO	unilateral hysterectomy-ovariectomy
US$	American dollar
WEI	weaning-to-estrus interval
Wn	weaning
Wn-7	the last 7 days before weaning
wt	weight
µg	microgram
µl	micro litre

Chapter 1: General introduction

One of the most important factors determining profitability at the primary production level of the pork industry is the number of pigs weaned per sow per year. This number is dependent on several factors. First, the number of litters produced per sow per year, which is dependent on gestation and lactation length, and weaning-to-estrus interval. The number of pigs weaned per litter depends on litter size and pre-wean mortality. Litter size at birth is in turn dependent on ovulation rate and prenatal survival. There has been a strong genetic selection for litter size, and total number of pigs born in a litter has increased in the past 20 years (Boulot *et al*., 2008). Unfortunately, the number of pigs born alive has not increased to the same extent, and the number of pigs weaned per litter has lagged even more (Figure 1-1 and Hoving, 2012). As a consequence, the number of stillborn pigs and pigs lost due to pre-weaning mortality have increased over time, which is economically wasteful. With the high feed prices of today's market, it is important to use energy as efficiently as possible, and any method to decrease pre- and post-natal losses should be considered.

1.1 N-3 LCPUFA supplementation to gilts and sows

Another factor influencing sow farm profitability is sow longevity. Increasing the time that a sow spends in the herd decreases the overall herd replacement costs. Reproductive failure is still one of the main reasons for culling in young sows (Lucia *et al*., 2000). Particularly, second parity sows seem to show suboptimal litter sizes and/or farrowing rates, which has been related to high body weight loss during first lactation (Schenkel *et al*., 2010; Hoving, 2012). A major research focus of the Swine Reproduction-Development Group (SRDP) at the University of Alberta has been the study of effects of maternal nutrition during lactation on subsequent reproductive performance (Zak *et al*., 1997a, b; Clowes *et al*., 2003; Vinsky, 2006) and this has resulted in the development of a research model to induce catabolism in the first lactation (described by Patterson *et al*., 2011). This research model utilizes feed restriction of sows during the last week of lactation to 60% of expected feed intake, with expected feed intake calculations based on previous studies and initial feed intakes of individual sows during early lactation. As well as being used to describe the effects of catabolism in first parity sows on subsequent

reproductive performance (Patterson *et al*., 2011), this research model has also been used to study the benefits of delayed breeding after weaning, either using "skip-a-heat" breeding or treatment with oral progestagens (Patterson *et al*., 2010). Both management practices negated the effects of lactational catabolism on subsequent reproductive performance, but resulted in an increase in the number of non-productive days, which is not ideal. As part of the research reported in Chapter 3 of this thesis, a different approach was taken to try and improve the performance of the catabolic, primiparous sow. Feeding first parity sows the marine-oil based supplement, Sow Fat Pack 10 (also called Gromega in the USA, and previously called Fertilium), has been shown to improve litter size in the past (Webel *et al.*, 2003; Spencer *et al.*, 2004) and was suggested to improve embryonic survival (Webel *et al*., 2004). The marine-oil based supplement used in this research is rich in omega-3 (n-3) long chain polyunsaturated fatty acids (LCPUFA) and additional benefits of n-3 LCPUFA supplementation on post-natal growth (Rooke *et al.*, 2000 and 2001b; Mateo *et al.*, 2009) and pre-weaning mortality of the litter (Rooke *et al.*, 2001a) have also been reported. However, as described in the literature review that comprises Chapter 2 of this thesis, the responses to LCPUFA supplementation were inconsistent. It, therefore, seemed appropriate to use the well-defined catabolic, primiparous sow model developed at the University of Alberta to further define situations in which positive effects of n-3 LCPUFA supplementation on reproductive performance and litter characteristics might be expected.

1.2 Litter birth weight phenotype

Besides the number of pigs weaned per sow per year and sow longevity, another important factor in farm profitability is the size and growth uniformity of pigs weaned. Uniformity in body weight at time of slaughter is critical for efficient use of all-in/all-out systems (Deen, 1997). Although the common practice is to sort pigs by size at entry to the nursery and/or grow-finish barns (Deen, 1997; Tokach, 2004), research has shown that this is not effective in decreasing within-pen weight variation at market (O'Quinn *et al*., 2001), and it increases aggressive behaviour during the 2 days after regrouping (O'Connell *et al*., 2005). The fact that sorting by size at entry to the grow-finish barn does not decrease within-pen weight variation at slaughter suggests that pigs have different growth potentials, which show up mostly in the later stages of the grow-finish phase. Growth after birth is mainly a result of an increase in muscle fiber size

(hypertrophy), as the number of muscle fibers remains constant after birth (as reviewed by Rehfeldt and Kuhn, 2006). This means that, to understand the differences in growth potential and work on the problem of body weight variation in the grow-finish barn, we must focus on the prenatal development of the pig, and specifically factors that can influence the development of muscle fibers (myogenesis). Several researchers have found effects of individual birth weight on growth performance up to slaughter (Quiniou *et al*., 2002; Gondret *et al*., 2006; Fix *et al*., 2010), which suggests that either, 1) individual birth weight has a direct effect on growth performance, 2) birth weight and growth performance are both the result of intra-uterine problems of fetal development, or 3) a combination of these factors determine post-natal growth performance. In contrast to effects of variation in individual birth weight on growth, the effects of litter average birth weight on growth rate have been much less researched.

The successful selection for increased litter size has resulted in decreased birth weight of piglets and an increase in within-litter variation in piglet birth weight (Quiniou *et al*., 2007, referenced by Foxcroft *et al*., 2009). As reviewed by Foxcroft *et al*. (2007) and shown in Figure 1-2 from Smit (2007), very large litters born to the most prolific sows will consistently show a lower litter average birth weight phenotype than that seen in the best sows with between 10 and 15 pigs total born: Overall, across the whole range of litter sizes, the total number of pigs born explains only a part of the variation in litter average birth weight ($R^2 > 0.2$) (see Figure 1-2).

However, although litter average birth weight can be 2 kg or higher in litters of 10 to 15 total pigs born, the variation in litter average birth weight between litters in this population (as high as 1 kg) is even greater than the variation of individual birth weight within litters (600 to 800 g): Furthermore, although the number of pigs born between 10 and 15 accounts for some of the variation in litter average birth weight (R^2 around 0.04), the variation in litter average birth weight is largely independent of litter size born (Table 1-1; Smit, 2007). This means that other mechanisms must play a role in determining litter average birth weight and particularly the low litter average birth weights seen in mature sow populations. As shown in Table 1-1, Smit (2007) showed that in litters between 10 and 15 pigs born in total, litters with a low average birth weight had more piglets born dead, and higher pre-weaning mortality, than did litters with a high average birth weight. As a result, the number of piglets weaned per litter was 1.35 piglets lower in litters with

low average birth weight compared to litters with high average birth weight, even though total number of pigs born was similar between the two groups. This shows that piglets from low birth weight litters were weaker overall.
As reviewed in more detail in Chapter 2, low average birth weight in litters, especially in higher parity sows, is hypothesised to be the result of a cascade of pre-natal events: it starts with high ovulation rates (>25 ovulations) and decent embryonic survival. This leads to intrauterine crowding (IUC) in early gestation. IUC causes placental development to be limited from d30 of gestation onwards, which then leads to measurable effects on fetal development by d50 of gestation onwards.

IUC can cause fetal programming to occur. Fetal programming results in a change in the number and type of muscle fibers, influencing the growth rate potential of piglets after birth (see reviews of Foxcroft *et al*., 2006; Rehfeldt and Kuhn, 2006). One can expect to see a higher proportion of low birth weight piglets in litters with low average birth weight, and so one may expect that on average this litter will have a compromised growth rate. However, IUC affects all piglets in a litter. Therefore, a piglet that weighed 1.5 kg at birth coming from a low average birth weight litter may still have a different growth potential compared to a piglet weighing 1.5 kg at birth that came from a high average birth weight litter and whose growth was limited by other intra-uterine events than early IUC of the whole litter. Their differences in growth potential may only become apparent during the later stages of the finishing period, as discussed above. It is, therefore, important to develop a better understanding of the different growth potential of pigs born in low and high average birth weight litters. This knowledge could help insure that all pigs are fed optimally according to their specific needs and are marketed at an optimal weight.

1.3 Aim, outline and hypotheses of this thesis

The objectives of the research described in this PhD thesis were: 1) to investigate effects of marine-oil based n-3 LCPUFA supplementation to gilts during later gestation and lactation on litter quality and growth performance and subsequent sow reproductive performance , 2) to investigate effects of marine-oil based n-3 LCPUFA supplementation to sows with a predicted low birth weight phenotype on litter quality, growth performance and carcass quality, 3) to investigate effects of litter average birth weight on

individual lean growth performance and carcass quality, 4) to investigate if litter average birth weight is a repeatable trait within sows, and 5) to better understand the biology of contemporary commercial sows.

The ultimate goal of the research performed as part of this PhD thesis was to improve the efficiency of the pork industry, by providing practical management tools to deal with low birth weight litters, as well as understanding the possible benefits of feeding marine-oil based n-3 LCPUFA to sows.

The following chapter of this thesis will give a comprehensive review of the existing literature concerning many of the processes described in this introductory chapter. It will start with a review of oocyte and embryo development, and factors affecting their quality. It will then describe placental development, and how disruptions in placental development can result in intra-uterine growth retardation and fetal programming of the fetus, with a focus on myogenesis. The second part of the review will give more background information on n-3 LCPUFA, their biosynthesis and metabolism, mechanisms of action, uptake of n-3 LCPUFA in the offspring through the placenta and milk, and effects of n-3 LCPUFA supplementation to sows on offspring performance.

In Chapter 3, the lactational feed restriction model as described above has been used as a background challenge against which to determine beneficial effects of marine-oil based n-3 LCPUFA supplementation to gilts on both lactation performance and subsequent reproduction. Offspring were followed until the end of the nursery period to measure effects of marine-oil based n-3 LCPUFA supplementation on growth rate of the offspring. It was hypothesized that marine-oil based n-3 LCPUFA supplementation to gilts during a part of gestation and during lactation would improve subsequent reproductive performance. Moreover, it was hypothesized that marine-oil based n-3 LCPUFA supplementation to gilts would increase growth performance of their offspring until the end of the nursery phase. Results of this trial showed little effect of marine-oil based n-3 LCPUFA supplementation on reproductive performance, but an increase in offspring growth performance.

Chapter 4 describes an experiment that investigated effects of high versus low litter average birth weight phenotype on post-natal lean growth performance, carcass quality,

and testicular development in male offspring. It was hypothesized that low birth weight litters would not grow as fast as high birth weight litters, and would have lower carcass quality. Moreover, low litter birth weight was hypothesized to result in lower testicular development. Repeatability of litter birth weight within sows was also investigated in Chapters 4 and 5, and it was hypothesized that litter birth weight phenotype was repeatable within sows. Results of this trial showed that low birth weight litters needed nine more days to reach the same market weight as high birth weight litters, due to lower average daily gain in pigs from low birth weight litters.

Considering the lower growth rate in low birth weight litters found in Chapter 4, and the increased growth potential of pigs born from sows supplemented with marine-oil based n-3 LCPUFA found in Chapter 3, it was suggested that feeding marine-oil based n-3 LCPUFA to sows that give birth to low birth weight litters, would increase the growth rate of their offspring, thereby decreasing the gap in growth rate between low and high birth weight litters. Therefore, to specifically look at the effect of marine-oil based n-3 LCPUFA supplementation on growth rate of litters with a low birth weight, a third research trial described in Chapters 5 and 6 was completed. It was hypothesized that feeding marine-oil based n-3 LCPUFA to sows during rebreeding, during gestation and during lactation would increase n-3 LCPUFA in sow serum, colostrum, milk and tissues of stillborns and would increase IgG concentration in sow serum and colostrum. Moreover, maternal marine-oil based n-3 LCPUFA supplementation was hypothesized to increase growth performance of low birth weight litters and improve carcass quality. Chapter 5 describes the effects of marine-oil based n-3 LCPUFA supplementation to all sows on trial on litter quality and growth until weaning, and it explores the interactions between marine-oil based n-3 LCPUFA supplementation and litter birth weight phenotype. Chapter 6 describes data from a subset of litters, which were selected for their low litter average birth weight. These litters were followed from birth, through the nursery and grow-finish periods, until slaughter, and carcass quality was assessed.

In the final chapter of this thesis, the findings from Chapters 3 to 6 are discussed and compared with existing literature presented in this thesis, to draw final conclusions, and to give practical recommendations. In addition, questions raised by the combined findings and potential future research are discussed.

Overall, the research completed during this PhD program formed part of a close collaboration with staff of JBS United Inc., located in Sheridan, Indiana, USA and the research trials described in Chapters 4, 5 and 6 were actually conducted at JBS United research facilities. Access to commercial sow populations for research purposes has become an integral part of the R&D continuum of the SRDP at the University of Alberta. The philosophy of integrating more basic research studies based at the intensive research facilities of the Swine Research & Technology Centre at the University of Alberta, with research in commercial sow populations of industry collaborators, was integral to the research program described in this thesis.

Table 1-1. Litter characteristics of litters with a low litter average birth weight (LBW) or low litter average birth weight (HBW), when all litter sizes are taken into account, or just litter sizes between 10 and 15 piglets born in total (Smit, 2007)

	All litter sizes			Litters of 10 to 15 total born		
	LBW	HBW	P-value	LBW	HBW	P-value
n (litters)	1619	1307		549	545	
Ave bw	1.18	1.83	< 0.001	1.18	1.76	< 0.001
Within-litter SD bw	0.284	0.265	< 0.001	0.272	0.287	0.01
# pigs born total	13.23	8.76	< 0.001	12.38	12.44	0.59
# pigs born alive	11.83	8.53	< 0.001	11.37	12.09	< 0.001
# pigs born dead	1.44	0.30	< 0.001	0.99	0.31	< 0.001
# pigs weaned	9.81	7.82	< 0.001	9.37	10.72	< 0.001

Ave: average, bw: birth weight, SD: standard deviation

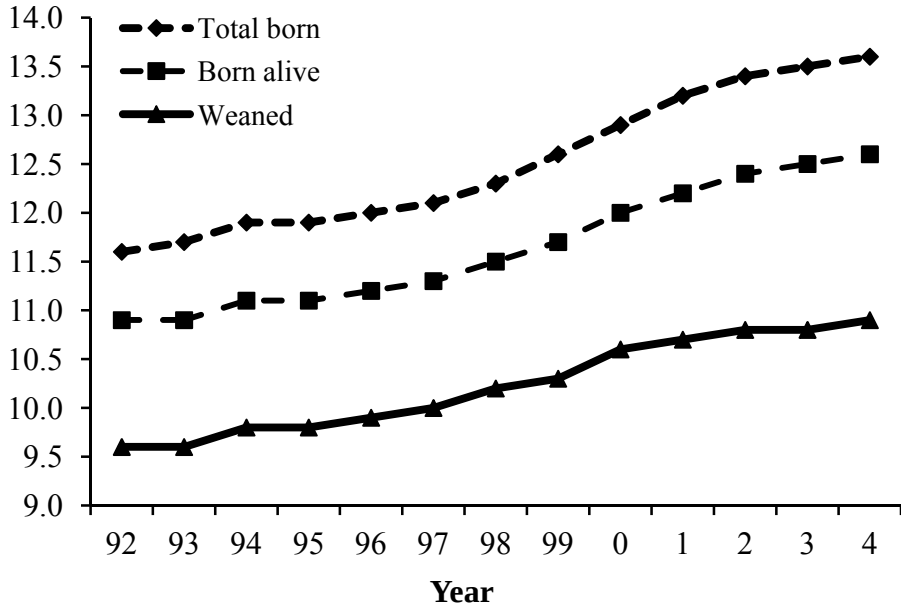

Figure 1-1. Evolution of litter size in French sow herds between 1992 and 2004. (Adapted from Boulot *et al*., 2008)

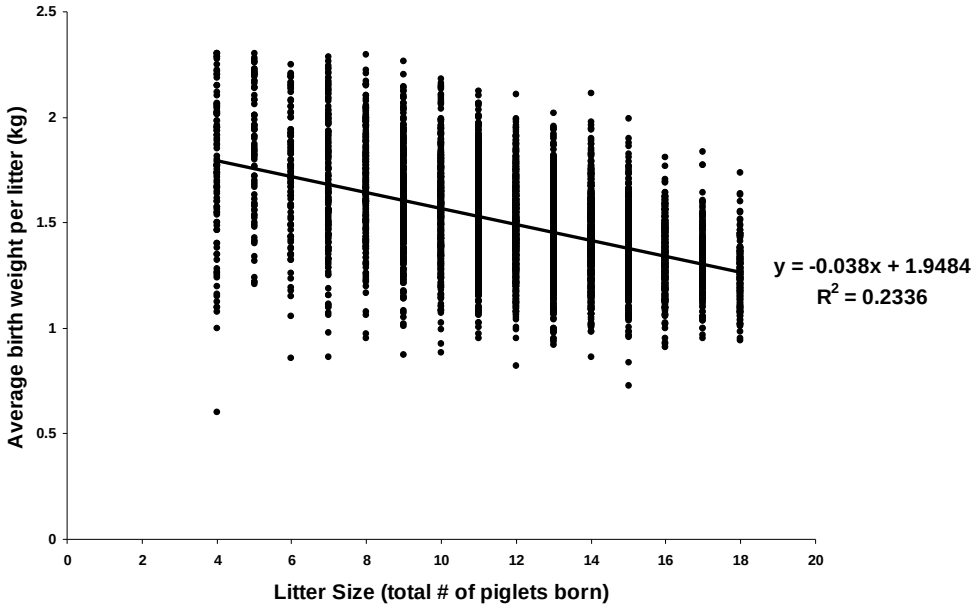

Figure 1-2. Relationship between litter size (as total number of pigs born) and litter average birth weight (n=5290) (Smit, 2007)

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Chapter 2: Literature review

This chapter will provide a review of topics that were briefly introduced in the previous chapter and provide the basis for the research described later in this thesis. This review is split into two main themes. The first describes prenatal development, the processes that can affect this development, and how in turn this may affect postnatal performance. The second part of the review will focus on the role of n-3 fatty acids as nutritional supplements, to provide an understanding of how n-3 fatty acid supplementation can affect reproduction and offspring growth performance.

2.1 Prenatal processes involved in litter size and quality

In the commercial pork industry, the number of offspring born is an important economical trait. For many years pigs have been successfully selected for increased litter size. Ideally, the pig industry wants big litters with a high birth weight and low within-litter variation in birth weight. Moreover, the growth potential and uniformity of pigs until slaughter is critical in the use of all-in/all-out grow-finish barns, and for the efficient marketing of pigs to meet specific carcass specifications (Deen, 1997). Both litter size and quality at birth depend on processes such as oogenesis, ovulation rate, fertilization rate, early embryonic survival (before day 30 of gestation) and fetal survival (after day 30 of gestation).

The first section of this review will focus on the different processes involved in litter size and quality at birth, and how prenatal events can have an impact on growth performance and carcass quality after birth.

2.1.1 Oogenesis and ovulation rate

In mammals, oogenesis, or oocyte maturation, occurs in four different phases during the female's life (Hunter, 2000). The first phase is mitotic division of primordial germ cells, which occurs prenatally. This is followed by nuclear arrest at the end of the first meiotic prophase, which occurs prenatally and remains in this stage through birth and into puberty. The third phase is cytoplasmic growth and development of the zona pellucida and junctional complexes (gap junctions) between the oocyte and neighbouring follicular cells, which occurs at the same time as follicular development from a primordial follicle

to a preovulatory follicle and takes around 3 months. The last phase consists of final nuclear maturation and resumption of meiosis, which occurs near the time of ovulation (Hunter, 2000).

In the pig, primordial follicles need more than 100 days to reach preovulatory status (Morbeck *et al*., 1992). During this time, many morphological and functional changes occur in the oocyte within the follicle and many interactions occur between the oocyte and its surrounding follicular cells through the gap junctions. This interaction between oocyte and follicular cells is needed for oocyte maturation (Moor *et al*., 1990) and the follicular cells are fundamental for the regulation of oocyte meiotic arrest and meiotic resumption, besides providing nutrients. Furthermore, oocyte development is also regulated by paracrine signals from the granulosa cells, and growth factors like insulin-like growth factor 1 (IGF-1) and epidermal growth factor (Hunter, 2000; Hunter and Paradis, 2009). Meiotic resumption is activated by the preovulatory luteinizing hormone (LH) surge. In pigs, the first meiotic division is completed in preovulatory follicles about 36-40h after the LH surge, coincident with ovulation. The oocyte then enters a second period of meiotic arrest until fertilization, which acts as the trigger for the second meiotic division and extrusion of the second polar body (Hunter, 2000).

Follicles need to have a certain diameter to be able to support oocyte maturation. Hunter (2000) mentioned that larger, more mature follicles produce better quality oocytes. However, even follicles of similar size can show differences in follicular fluid steroid concentrations, granulosa cell number and LH receptors which persist up to the time of ovulation and beyond, and have been associated with differences in the luteinisation response and in oocyte maturation (Hunter and Wiesak, 1990). The follicle can therefore have a critical influence on oocyte maturation and can change oocyte quality. In turn, follicular and oocyte development have consequences for embryo development and survival, because the maturational state of oocytes before ovulation is associated with their development as early zygotes (Xie *et al*., 1990a) and errors in oocyte maturation can have a major impact on embryonic development.

2.1.2 Early embryonic survival

In well managed sow herds, fertilization rate is considered to be close to 100%. After fertilization, the male and female pronuclei fuse into a single diploid nucleus. The zygote then goes through several cleavage stages and mitotic divisions to become a blastocyst, with an inner cell mass, a cavity called the blastocoele and a single layer of extra-embryonic cells called the trophoblast. The blastocyst then expands and eventually hatches from the zona pellucida. The embryo is then called a hatched blastocyst and in the pig is initially unattached in the uterine lumen (Dantzer, 1985). The development from zygote to hatched blastocyst takes around 6 to 7 days (Stroband and Van der Lende, 1990). As referenced by Stroband and Van der Lende (1990), around day 12 of gestation, hatched blastocysts start to elongate and they also start to produce estrogens, prostaglandins and proteins. These secretions may be involved in maternal recognition of pregnancy, which also takes place during the elongation process of blastocysts.

Both the embryo and the uterus undergo dramatic changes from the time of fertilization to the time of implantation around day 12-16. Until implantation, the embryo depends on the uterine environment which must provide all the necessary developmental components, like hormones, amino acids, proteins, carbohydrates, glycosaminoglycans and lipids (reviewed by Pasternak, 2012). This is a vulnerable period for embryonic development, and not all embryos survive. In fact, in the pig, preimplantation embryonic losses are generally considered the largest proportion of prenatal loss, with lower losses in the postimplantation period (as reviewed by Ashworth and Pickard, 1998). The next section will discuss several factors related to early embryonic survival

2.1.3 Factors affecting early embryonic survival

One of the major contributors to early embryonic loss, according to Pope (1988), is asynchrony among embryos. For example, Xie *et al*. (1990b) reported that later ovulating oocytes developed into the smallest embryos and were more vulnerable to changes in the uterine environment. Subsequently, Zak *et al*. (1997a,b) reported that a catabolic state in sows immediately before weaning had detrimental effects on embryonic survival and that this was apparently mediated by differences in the physiological state of the follicle and the maturational state of the oocyte. On the other hand, Dziuk (1987) suggested that asynchrony between embryos may be due to an increased period from fertilization of the

first oocyte to fertilization of the last oocyte. The fertilization process can take up to 8 to 10 hours and the variation of development may persist to the critical times at implantation and establishment of pregnancy. Not all embryos develop at the same rate, possibly due to the above mentioned factors. As reviewed by Pope (1988), most pig blastocysts are in themselves viable, but the less developed blastocysts are more susceptible to an advanced uterine environment than are blastocysts that are morphologically more mature. Dziuk (1987) suggested that it is possible that the stage of uterine development is not in synchrony with the stage of development of all embryos. This is intrinsic to the success of the embryo-maternal interaction, because an embryo that responds at an inappropriate stage of uterine development, will not survive. However, it seems that embryos can change their own environment by delivering steroids to the uterine environment, thereby influencing local vascular permeability and endometrial protein release (as reviewed by Stroband and Van der Lende, 1990). One of the steroids released by the embryo is estrogen, which seems to change uterine secretions and advancing the uterine environment (Geisert *et al*., 1982). This could be the main reason why variation in developmental stage of embryos can have such a detrimental effect on the slower developing embryos.

Lastly, asynchrony between embryos has been suggested to have an epigenetic component (Geisert and Schmitt, 2002) and it is possible that maternal feed restriction in lactation can directly influence embryonic survival through this route (Foxcroft *et al*., 2007). Indeed, Vinsky *et al*. (2007) showed that a sub-population of embryos within litters from nutritionally restricted sows were epigenetically affected and lost before day 30 of gestation.

As already mentioned above, maternal nutrition is able to affect embryonic survival, and it can do this through several routes. For example, maternal nutrition during lactation in sows, around ovulation or during early pregnancy in gilts, can have an effect on plasma progesterone concentrations and this in turn has been associated with increased embryonic mortality in pigs (Pharazyn, 1992). Also, Hoving (2012) showed that sows with high weight loss during lactation reached the progesterone peak values later than sows with a low body weight loss, and that high body weight loss was associated with lower embryonic survival. Other studies have confirmed that maternal nutrition during late lactation can have a profound effect on embryonic quality and survival in the subsequent litter (Zak *et al*., 1997a, b; Clowes *et al*., 2003; Patterson *et al*., 2011). It is

likely that the nutritional effect on embryonic survival and/or quality is at least in part due to changes in oocyte quality which can be mediated in two ways; firstly, directly, through influencing follicular development and the follicle environment and secondly, indirectly, through changes in gonadotrophin secretion (Hunter, 2000; Zak *et al*., 1997a, b). Besides embryonic losses, gross changes in body weight and body condition have also been associated with changes in the weaning-to-estrus interval, the proportion of sows returning to estrus, and pregnancy rates (Kirkwood *et al*., 1987; Mullan and Williams, 1989).

The lactating sow has a high requirement for energy and protein and approximately 75% of the energy requirement goes to milk production (Aherne and Kirkwood, 1985). Within certain limits, a reduction in feed intake during lactation is compensated by an increased mobilization of body reserves so as to maintain the milk production (Mullan and Williams, 1989; Pluske *et al*, 1998; Patterson *et al*., 2011). Since the reproductive tissues have a low priority for nutrients, the decrease in feed intake or the availability of nutrients will have more profound effects on the reproductive system as compared to other physiological functions. Lactating primiparous sows are especially prone to becoming catabolic, because they are still growing themselves and tend to have a lower voluntary feed intake than older sows. This impacts embryonic survival in the subsequent litter, resulting in the smaller litter sizes commonly referred to as the second parity dip (Hoving 2012 and reviewed by Soede and Kemp, 2013). However, it has also been shown recently that modern sows seem to react differently to restricted feeding challenges in late lactation, decreasing embryo weight rather than increasing the weaning-to-estrus interval or decreasing embryonic survival (Patterson *et al*., 2011).

Ashworth *et al.* (1999) showed that gilts that were fed 2.8 x maintenance during the estrous cycle preceding breeding had a higher percentage of embryos that survived; recovered blastocysts were also larger and had enhanced metabolic and secretory activity *in vitro* compared to embryos from gilts fed at maintenance. Moreover, gilts fed 2.8 x maintenance showed less within-litter variation in development, which may provide a means of reducing within-litter variability in birth weight and vitality (Ashworth *et al*., 1999).

Yang *et al*. (2000a) showed that sows that were fed a low lysine diet had fewer large follicles and more medium-sized follicles compared to sows fed medium or high lysine diets. However, there was no difference between sows fed the medium and high lysine diets, which suggests that above a certain threshold level of nutrient/protein intake, quality of follicles is not affected by further increasing nutrient intake during lactation (Yang *et al*., 2000a). Also, Clowes *et al*. (2003) showed that ovarian function was suppressed in sows that had mobilized the most body protein, that follicle size was smaller and that the follicles contained less follicular fluid and had lower estradiol and IGF-I contents. This suggests that inadequate nutrient intake during lactation retarded growth rate and development of follicles. However, there was no difference between sows fed the medium and high lysine diets, which suggests that above a certain threshold level of nutrient/protein intake, quality of follicles is not affected by further increasing nutrient intake during lactation (Yang *et al*., 2000a). In another study, Yang *et al*. (2000b) showed that sows fed a low lysine diet had less frequent LH pulses than sows fed medium and high lysine diets. This was in accordance with findings of Zak *et al*. (1997a, b) that showed nutritionally induced catabolism can result in total suppression of pulsatile LH secretion during lactation, thus influencing follicle development and oocyte maturation.

Generally, ovulation rate may be increased by increasing the size of the preovulatory pool of follicles, whereas embryonic survival may be increased by improving follicle and oocyte quality (Yang *et al*., 2000a). Generally, nutrient deficits during lactation did not affect ovulation rate (Yang *et al*., 2000a; Aherne and Kirkwood, 1985; Kirkwood *et al*., 1987), although Zak *et al*. (1997) did report a decrease in ovulation rate after imposing relatively severe restricted feeding (50% of expected intake) during late lactation. They suggested that this is because modern genotype sows tend to show little increase in weaning-to-estrus interval, even after experiencing severe catabolism in lactation. Therefore, follicles mature and ovulate in a relatively adverse environment, which may reduce the size of the recruited pool of preovulatory follicles. However, more recently, using a similar level of feed restriction in primiparous sows, but with a shorter lactation and a different genotype, Patterson *et al*. (2011) did not see an effect of feed restriction on weaning-to-estrus interval, nor on ovulation rate. Furthermore, the extent to which the sow mobilized body tissues to meet the energy costs of milk production was associated with the degree to which litter weaning weight and/or embryonic development was

affected, with a category of "Risk" sows identified that used body tissues to maintain milk production at the expense of embryonic growth in the subsequent litter.

This body of research clearly shows that, although responses of sows may differ, maternal nutrition during lactation and/or the rebreeding period is a key factor in reproductive outcome, as seen by the effects on early embryonic survival and quality.

2.1.4 Fetal survival

Until recently, it was thought that the largest proportion of prenatal loss occurred before the implantation period. Johnson *et al*. (1999) selected pigs for ovulation rate and embryonic survival by counting the corpora lutea and surviving fetuses at day 50 of gestation via laparotomy. Selection increased ovulation rate dramatically, but embryonic survival decreased. Bazer *et al*. (1969) concluded that increased embryonic loss, associated with a greater number of embryos in the uterus, was due to maternal limitations and not to inherent limitations of the embryo. More recently, Rosendo *et al*. (2007) selected two lines during six generations for high ovulation rate at puberty (OR-line) or high prenatal survival corrected for ovulation rate in the first two parities (PS-line). The total number of piglets born did not change in the OR-line, but significantly improved in the PS-line (0.24 ± 0.11 piglets per generation), which shows that selection for both ovulation rate and embryonic survival is a better approach than selecting for ovulation rate alone. Because of high ovulation rates in higher parity sows (Vonnahme *et al*., 2002; Town *et al*., 2005) and modest to good embryonic survival in the pre-implantation period, the number of embryos surviving to the immediate post-implantation period (day 25 to 30) frequently exceeds uterine capacity (Foxcroft *et al*., 2006). The result is intrauterine crowding and an increase of prenatal loss in the post-implantation period. Due to this increased fetal loss, the total prenatal loss in sows with high ovulation rates increases to 40-60%, compared to 10-35% for sows with lower ovulation rates (Foxcroft *et al*., 2006). This observation is consistent with the earlier conclusion of Johnson *et al*. (1999) that selection for uterine capacity might be the best approach in genetic selection programs. Uterine capacity can be defined as 'the physiological and biochemical limitations imposed on conceptus growth and development by the uterus' (Bazer *et al*., 1969), which in practical terms defines the number of embryos that the pig uterus can successfully carry to term (Ford *et al*., 2002).

2.1.5 Placental development

It is important to understand the mechanisms involved in fetal growth (increase in number and size of cells or in the mass of tissues) and development (changes in structure and function of cells or tissues) (Wu *et al*., 2006). Different factors influence fetal growth and development, many of which act upon the placenta, changing its size or functional capacity and thus the availability of nutrients for fetal growth. The placenta provides the site of nutrient and oxygen transfer from the mother to the young, and waste products from the young to the mother. The placenta also acts as an immunological barrier, preventing maternal rejection of the fetal allograft (Beer and Sio, 1982). Moreover, the placenta is an endocrine organ, synthesizing and secreting several hormones, growth factors, cytokines and other bioactive substances. The pig has a diffuse placenta, which has microvilli that are distributed more or less evenly over the entire surface of the chorionic sac. Therefore, exchange between mother and fetus can occur over almost the entire surface of the chorion (Senger, 2005). The exchange of nutrients occurs through three different mechanisms. The first is direct transfer of nutrients from maternal to fetal plasma, the second consists of placental metabolism and nutrient consumption, and the third uses placental metabolism of nutrients to alternate substrate forms (Père, 2003). Nutrient supply to the fetus depends on placental size, morphology, blood supply and transporter abundance. The placenta goes through several physiological changes during pregnancy, regulated by hormones, nutrient-related genes, and angiogenic factors (Belkacemi *et al*., 2010). Angiogenesis is defined as the development of new vascular structures and involves the branching of new microvessels from pre-existing larger blood vessels (Barut *et al*., 2010). In pigs, the initiation of angiogenesis occurs around day 10 to 14 of gestation, at the same time as implantation, in response to the production of conceptus estrogen. There are two waves of placental angiogenesis during gestation in the pig; one in the peri-implantation period (day 15 to 20), and a second wave which starts around day 50 of gestation (as reviewed by Tayade *et al*., 2007). Changes in the placental structure or function may contribute to altered nutrient supply, and this may lead to changes in fetal growth.

2.1.6 Causes of intra-uterine growth retardation

Because intrauterine growth retardation (IUGR) in humans is one of the most important perinatal syndromes and is a worldwide problem, it has been well studied in humans

(Raghupathy *et al*., 2012). Therefore, information of human IUGR will be discussed and where possible, will be linked to research findings in pigs. Fetal growth retardation in humans may occur through two distinct growth patterns; the first one is that of symmetrical IUGR, where the fetus grows at a constant but slower rate than normal, which is typical of a hereditary (intrinsic) limitation in growth potential; the second one is asymmetrical, where the rate of growth slows or even stops entirely. This type of growth retardation results in brain sparing; growth of the brain is relatively preserved, while growth of the liver, spleen and somatic tissues are affected, thus resulting in disproportionate body measurements. The brain:liver weight ratio is thus considered a good measurement of IUGR in mammals (Cooper, 1975). Asymmetrical fetal growth retardation is most likely caused by factors affecting placental function, especially decreased maternal supply of nutrients or oxygen to the placenta, or decreased placental substrate transfer. Therefore, it is likely that detrimental effects on placental development will be the earliest sign of IUGR, as seen in the pig by Knight *et al*. (1977 and Town *et al*. (2005).

In humans, a common cause of IUGR is placental ischemia. Ischemia is a restriction in blood supply, causing a shortage of oxygen and glucose needed for cellular metabolism. It has been shown that placental oxygenation is important in the control of fetoplacental angiogenesis and thus in villous differentiation (Kaufmann *et al*., 2004). Abnormal angiogenesis may then lead to IUGR. Indeed, Barut *et al*. (2010) showed that IUGR placentae have widespread infarct areas and concluded that a change in placental development accompanying deteriorations in angiogenesis happens in IUGR. Although the clinical signs of ischemic placental disease can only be seen in the second half of pregnancy, the pathophysiological processes initiating the disease originate in the first half of the pregnancy (Kinzler and Vintzileos, 2008). This is similar to research in pigs, that showed that relative intra-uterine crowding in early gestation (up to day 30 of gestation) resulted in lower placental weights already at day 30 of gestation, while it did not yet affect fetal weight. However, at day 50 of gestation (Patterson *et al*., 2008) and day 90 of gestation (Town *et al*., 2004) both placentae and fetuses were smaller when experiencing early intra-uterine crowding. Although the placenta seems to have a certain ability to compensate for its smaller size by increasing the width of the microscopic folds, thereby potentially improving nutrient transfer from the sow to the fetus (Vallet and

Freking, 2007), this compensation does not seem to be enough to offset the negative effects of intra-uterine crowding on fetal growth.

Fetal nutrient availability depends on maternal food intake, availability of nutrients in the maternal circulation, and the ability of the placenta to transport substrates to the fetal circulation (Belkacemi *et al*., 2010). The maternal diet during gestation controls fetal growth directly by providing glucose, amino acids and other essential nutrients for the conceptus. This has an effect on fetal development and piglet birth weight (Robinson *et al*., 1999). When maternal nutrition was increased from day 25 to 50 of gestation, the greatest impact could be seen on the smallest pigs within a litter (Dwyer *et al*., 1994). This suggests that low birth weight is caused by relative under-nutrition of the smallest fetuses in the uterus. Indeed, placental weight is correlated with dietary intake in mammals, although specific effects of maternal nutrition on placental mass depends on the timing, duration and etiology of nutritional restriction (Belkacemi *et al*., 2010). Maternal under-nutrition during gestation often results in some level of placental vascular and angiogenic dysfunction. For example, when pigs were fed a diet restricted in proteins for up to 60 days of gestation, placental arginine, a common substrate for nitric oxide (NO), NO synthesis and NO synthetase activity all decreased (Wu *et al*., 1998). NO is a mediator of angiogenesis and plays a role in modulating vascular resistance (Belkacemi *et al*., 2010 and references therein). Also sheep restricted to 50% of normal feed intake between 28 and 78 days of gestation had lower levels of NO in maternal-fetal plasma (Wu *et al*., 2004). As arginine is a common substrate for NO, it has been suggested that feeding L-arginine to pigs improves placental function. Indeed, Hazeleger *et al*. (2007a and b) reported increased vascularization in the placenta of gilt conceptuses, and more surviving embryos, as a result of L-arginine treatment in early gestation. Moreover, Mateo *et al*. (2006) found that supplementing gilts with L-arginine-HCL increased the number of live-born pigs and total litter weight, and also Ramaekers *et al*. (2006) showed positive effects of feeding L-arginine from d 14 to 28 of gestation on litter size, farrowing rate, and within-litter standard deviation in birth weight. Recent studies showed that there was an increased embryonic growth rate and higher expression of the angiogenin gene in placentae of L-arginine-supplemented compared to control sows, confirming the potential for L-arginine supplementation to improve placental angiogenesis in early gestation (Novak *et al*., 2012). However, Zier-Rush did not find positive effects of feeding L-arginine in early gestation on number of pigs born total or farrowing rate, and L-arginine

supplementation in late gestation did not improve birth weight. Thus, timing and amount of supplementation seem to be crucial to show positive effects.

Another mechanism regulating placental nutrient transfer to the fetus is imprinting of genes. Imprinted genes are only expressed from either the maternal or the paternal allele and imprinting is suggested to be an epigenetic phenomenon (Burns *et al*., 2001), with DNA methylation as a key molecular mechanism of imprinting. The imprinted genes are marked differently in egg and sperm by changes in their methylation state, and inheritance of these epigenetic marks leads to differential gene expression. The exact mechanism of genomic imprinting was extensively reviewed by Reik and Walter (2001) and imprinting is thought to influence the transfer of nutrients from the mother to the fetus and the newborn. Many of the identified imprinted genes are expressed in the placenta, are crucial for fetal development, and thus can determine litter size and birth weight. Fetal growth seems to be enhanced by paternally expressed genes and suppressed by maternally expressed genes (Reik and Walter, 2001). Imprinting can be disrupted in the early embryo by environmental influences, such as maternal nutrition, and inherited through many cell cycles into adult tissues (Young, 2001). This programming might also be maintained for several generations. For example, prenatal programming of birth weight by maternal food restriction or maternal exercise has been shown to last for more than one generation (Duttaroy, 2006). Stewart *et al*. (1975) also showed that when rats were exposed to a protein-deficient diet over twelve generations, it took three generations to normalize growth and development when refeeding with a normal diet.

Other factors that affect fetal growth are maternal maturity (physiological status of the uterus), environment (including maternal nutrition) and intrauterine crowding, which all have an indirect effect on fetal growth. The genes of the parents have a direct effect on fetal growth by providing the genetic potential of the fetus, and an indirect effect on fetal growth by influencing placental growth through imprinted genes. The epigenetic state (genomic imprinting) of the oocyte or the embryo also has a direct effect on fetal growth (Vinsky 2006) and an indirect effect via imprinted genes in the placenta as described above (Young 2001). The epigenetic state, affected by changes in DNA-methylation, can in turn be influenced by environmental factors, like maternal under-nutrition (Vinsky *et al*., 2006). The factors influencing placental growth and fetal growth are summarized in Figure 2-1.

2.1.7 Consequences of intra-uterine growth retardation

If the transfer of nutrients from mother to fetus is insufficient, fetal programming may occur, with consequences for fetal growth and growth potential after birth. Fetal, or prenatal, (re)programming is a response to the nutrient and hormonal milieu of a conceptus during 'critical periods' of gestation, which may alter expression of the fetal genome with lifelong, and sometimes generational, consequences (Lucas, 1991). David Barker was the first to recognize that adulthood diseases may have an origin in prenatal development. He and his colleagues showed a strong geographical relationship between coronary heart disease rates in adults and infant mortality rates, and showed that low birth weight was the most frequent cause of infant death in the period studied (Barker and Osmond, 1986). Using detailed birth records of the county of Hertforshire, England, Barker (1995) revealed strong inverse associations between birth or infant weights and death from coronary heart disease. This has led to the hypothesis that the metabolic, physiological or neuroendocrine adaptations that allow the fetus to survive a period of intrauterine deprivation results in a permanent reprogramming of developmental patterns of proliferation and differentiation events within key tissues and organs and pathological consequences in adult life (Barker, 1999). This hypothesis was originally called the 'Barker Hypothesis', but is now renamed as 'The developmental origins of health and disease hypothesis' or 'developmental programming'. It has now been recognized that many other disorders are included in this hypothesis, like obesity, type-2 diabetes, hypertension, stroke, the metabolic syndrome, and even immune and autoimmune disorders and behavioral problems (Phillips, 2006; Reynolds and Caton, 2012). Moreover, Lumey (1998), who studied effects of the Dutch famine during World War II on birth weight and adult diseases, has suggested modifying the hypothesis to recognize that "long term health effects after fetal under-nutrition may occur in the absence of a birth weight effect, and may not be apparent even in its presence". This shows that birth weight and fetal programming are two resultants of the same insult, or multiple insults, but are not necessarily linked to each other. Figure 2-2 shows several factors causing IUGR and fetal programming.

"The theory of fetal programming can be extended to include fetal origins of postnatal growth retardation, reduced feed efficiency, and reduced meat quality. This concept of fetal programming has far-reaching implications for the animal sciences" (Wu *et al.*, 2006). Additionally, IUGR has been associated with increased risk of neonatal mortality

and morbidity, another important aspect of livestock production (Reynolds and Caton, 2012). As shown in Figure 2-2, fetal programming occurs when nutrition of the fetus is insufficient. This means that all the factors affecting placental and fetal growth, as described above, are plausible candidates of fetal programming. One of those factors in polytocous species includes intrauterine crowding. During the implantation period of embryos, intrauterine competition occurs for the establishment of adequate surface area for nutrient exchange between the fetus and the mother (Foxcroft *et al*., 2006). Based on studies of early embryonic mortality in pigs, Dziuk (1968) suggested that when the number of embryos exceeds 14, intrauterine crowding is a limiting factor for litter size born and Knight *et al*. (1977) defined day 30 to 40 of gestation as the critical period when uterine capacity exerts these effects. However, Town *et al*. (2005) reported that intrauterine crowding also affects development of the surviving fetuses (IUGR) when more than nine fetuses were present *in utero* at day 30 of gestation. In pigs, this fetal programming has resulted in altered fetal organ development and a change in the number and type of muscle fibers, influencing the growth rate potential of piglets after birth (see reviews of Foxcroft *et al*., 2006 and Rehfeldt and Kuhn, 2006).

2.1.8 Fetal programming of muscle development

Muscle development (myogenesis) in the pig is a biphasic event. First, primary muscle (myo)fibers are formed between day 25 and 50 of gestation by the rapid fusion of primary myoblasts; then, secondary myofibers are formed between day 50 and 90 of gestation at the surface of primary myofibers (Figure 2-3). In late gestation, over 20 secondary myofibers occur around each primary myofiber. The increase in myofiber number (hyperplasia) ceases by day 90 of gestation; thus the number of primary and secondary myofibers formed at that point determines the total number of myofibers at birth. As myocytes and adipocytes are derived from a common mesenchymal precursor, excess adipose tissue is being developed at the expense of skeletal muscle when embryonic myogenesis is impaired (Wu *et al*., 2006 and references therein).

Wigmore and Stickland (1983) showed that the number of secondary myofibers and the total number of myofibres formed in the smallest fetuses in a litter (excluding runts) is lower than that of the largest fetuses in the same litter. Moreover, primary myofibres in small fetuses were smaller than in large fetuses. The total number of muscle fibers at

birth determines the postnatal growth potential of the pig. Indeed, Handel and Stickland (1988) showed that only piglets with high myofiber numbers were able to show catch-up growth postnatally, and Dwyer *et al*. (1993) showed that postnatal growth and feed conversion efficiency were positively correlated with myofiber number, and not with birth weight. It is believed that variation in primary myofiber number is more genetically controlled, and thus less affected by maternal status, than secondary myofiber number (Wigmore and Stickland, 1983). Nissen *et al*. (2004) suggested that this is because primary myofibers develop at a time where nutrients are not limiting to development, while secondary myofiber numbers are developing later, and can thus be affected by both genetic and environmental factors, such as nutrient supply. However, Rehfeldt and Kuhn (2006) have suggested that the number of primary myofibers might also be affected by maternal nutrition.

After birth, myofiber size increases by the addition of nuclei from muscle satellite cells, and subsequent hypertrophy of existing myofibers. No new myofibers can be formed after birth, and thus factors affecting fetal myofiber development can have a permanent, irreversible impact on muscle structure and postnatal growth potential (Du *et al*., 2011). Both maternal nutrient restriction during pregnancy (Du *et al*., 2010) and intrauterine crowding (Bérard *et al*., 2010) have been shown to affect myofiber numbers in offspring. Town *et al*. (2004) also showed that moderate intrauterine crowding resulted in lower numbers of secondary myofiber numbers, and found lower placental weights for litters experiencing crowding. They suggested that the effects of early intrauterine crowding on myofiber development happen by limiting placental development, which has detrimental effects on fetal development later in gestation, including myofiber development (Figure 2-3). It has been suggested that myofibers are especially vulnerable to nutrient availability during development because of their lower metabolic demands compared with tissues such as the brain, gut and placenta, which gives myofibers a lower priority in terms of nutrient partitioning during fetal development (Barcroft, 1946, referenced by Reynolds and Caton, 2012).

2.1.9 Myofiber numbers, birth weight and postnatal performance

The relationships between myogenesis, birth weight, growth and carcass quality were reviewed by Rehfeldt and Kuhn (2006). They showed that piglets with a low birth weight

had formed less myofibers during fetal development, mainly due to lower secondary myofiber numbers, and that they grew slower postnatally compared with piglets with a high birth weight. The authors concluded that the majority of low birth weight piglets have low numbers of myofibers differentiating during myogenesis and that myofiber number is important in determining birth weight. Moreover, low birth weight pigs have larger myofibers at slaughter, because of faster fiber growth, and they have more giant fibers than high birth weight pigs. Extreme fiber size and higher amounts of giant fibers are associated with poor meat quality in pigs. Also carcass weights, meat percentages and loin muscle areas were lower in low birth weight pigs, while fat percentage was higher than in high birth weight pigs. The authors concluded that "the data indicate that pigs of low birth weight develop lower carcass and meat quality and that this is related to low numbers of muscle fibers that undergo accelerated hypertrophy during postnatal growth" (Rehfeldt and Kuhn, 2006, and references therein). Since this review, new evidence has emerged that supports this conclusion. Gondret *et al*. (2006) showed that lean meat content at slaughter and weights of the *Longissimus* and *Semitendinosus* muscles were lower in low vs. high birth weight pigs. Moreover, low birth weight pigs had fatter carcasses, greater mean myofiber cross-sectional areas, and less total myofiber numbers than pigs with high birth weights. Tristán *et al*. (2009) found that compared to piglets weighing more than 2 kg at birth, piglets weighing less than 1.1 kg at birth had lower total cross-sectional area of the *Semitendinosus* muscle, as well as lower size and number of myofibers. Moreover, the low birth weight piglets still weighed less at weaning and post-weaning (67 days). Beaulieu *et al*. (2010) showed that low birth weight pigs tended to have lower number of secondary myofibers than high birth weight pigs, although they found limited effects of birth weight on carcass quality traits, only showing higher intramuscular fat levels for lower birth weight pigs. Alvarenga *et al*. (2012) did show lower hot carcass weights, meat content in the carcass and yield of ham, shoulder and belly in low birth weight pigs, as well as lower percentage of myofibers and higher percentage of connective tissues in the *Semitendinosus* muscle compared with high birth weight pigs.

Bérard *et al*. (2010) used the method of unilateral hysterectomy-ovariectomy (UHO) to look at the effect of more extreme intrauterine crowding on myofiber development. Interestingly, they discovered that intrauterine crowding reduced hyperplasia of both secondary and total myofibers in the dark portion of the *Semitendinosus* muscle and in

the Psoas Major muscle, and that this effect was independent of piglet birth weight and gender. In a follow-up study, this lab compared litters of UHO sows (resulting in higher intrauterine crowding compared to normal sows) with sows that had undergone unilateral oviduct ligation (resulting in lower intrauterine crowding compared to normal sows) (Pardo *et al.,* 2013). They noticed that the reduction of the *Semitendinosus* muscle weight relative to birth weight was, at 39%, slightly larger than for the other organs in crowded compared with uncrowded litters. Results from this study suggest that intrauterine crowding not only resulted in impaired myofiber hyperplasia, but also reduced hypertrophy of myofibers (Pardo *et al*., 2013). Again, this study did not show an interaction between surgical treatment and piglet birth weight, meaning that birth weight per se is not a factor for lower myofiber numbers, but that this is due to fetal programming of myogenesis during fetal development. This also means that intrauterine crowding can affect the postnatal growth performance of the entire litter, and not just the small pigs in that litter. This gives rise to the question how to identify litters at birth that underwent intrauterine crowding, which is important both for management practices, as discussed in Chapter 4, and in a research setting, when testing effects of intrauterine crowding on postnatal performance. An explanation of our experimental design will be explained in Chapter 4 and specific effects of litter birth weight on postnatal growth performance and carcass quality will be further discussed. Some other experimental designs and their limitations will be discussed in Chapter 7.

2.2 Omega-3 fatty acids

The second part of this review will focus on fatty acids, with a special focus on omega-3 (n-3) fatty acids. The biosynthesis and metabolism of n-3 fatty acids, their mechanisms of action, and dietary information will be discussed. Fatty acid transfer in the placenta and effects of maternal nutrition on fatty acid composition of fetuses will also be discussed.

2.2.1 Omega-3 fatty acid biosynthesis and metabolism

Fatty acids are hydrocarbon chains with a methyl group on one end and a carboxyl group on the other end. When a double bond between two C atoms in the hydrocarbon chain is present, the fatty acid is referred to as a mono-unsaturated fatty acid (MUFA) and with two or more double bonds as a poly-unsaturated fatty acid (PUFA). If the PUFA contains 20 or more C atoms in the hydrocarbon chain, it is referred to as a long-chain PUFA

(LCPUFA). Omega-3 (n-3) and omega-6 (n-6) fatty acids are both PUFAs that can be distinguished from each other by the location of the first double bond, counting from the methyl group end; n-3 PUFA have their first double bond on the third carbon atom, while n-6 PUFA have their first double bond on the sixth carbon atom. The simplest n-6 PUFA, linoleic acid (LA, C18:3 n-6), and the simplest n-3 PUFA, α-linolenic acid (ALA, C18:3 n-3), are both considered essential fatty acids. This is because mammals cannot synthesize them, and because they have been shown to be essential for normal growth and development. Thus, they need to be obtained through the diet (Russo, 2009). Absorption of non-esterified fatty acids in the diet is very efficient in mammals (Burdge, 2006). After absorption, fatty acids enter cells via fatty acid transporters, and are then converted to fatty acid acyl-CoA thioesters (Russo, 2009). At that point, they can be used in three different pathways; first, they can be used in ATP production by the classical β-oxidation pathway; second, they can be used as substrate for the synthesis of lipids, such as triglycerides and phospholipids; third, they can be metabolized further to obtain LCPUFAs (Russo, 2009), by several steps of desaturation and elongation (see Figure 2-4), which mainly occurs in the liver (Calder and Yaqoob, 2009). The first step is desaturation by Δ6-desaturase, which is the rate limiting step, followed by elongation and another desaturation by Δ5-desaturatase (Figure 2-4). This yields the n-6 LCPUFA arachidonic acid (AA) and the n-3 LCPUFA eicosapentaenoic acid (EPA). A pathway for further conversion of EPA to docosahexaenoic acid (DHA) involves two elongation steps, followed by desaturation by Δ6-desaturase, and translocation from the endoplasmic reticulum to peroxisomes for limited β-oxidation (Figure 2-4; Russo, 2009; Calder and Yaqoob, 2009). LA and ALA compete with each other for the use of the enzymes needed for desaturation, however Δ6-desaturase has a higher affinity for LA than ALA, meaning that the pathway leading to AA is preferred (Russo, 2009 and references therein). The conversion of ALA to EPA, DPA and DHA is poor in mammals, with very limited conversion all the way to DHA (Burdge and Calder, 2006). Therefore, EPA and DHA should be present in the diet, and one could argue that especially DHA is thus an essential fatty acid as well (Muskiet *et al*., 2004).

2.2.2 Eicosanoid production

Dihomo-γ-linolenic acid (DGLA), AA, EPA and DHA are all substrates for eicosanoid biosynthesis, using the same 2 types of enzymes, cyclooxygenases (COX) and

lipoxygenases (LOX) to produce prostanoids (prostaglandins, prostacyclins and thromboxanes) and leukotrienes (Figure 2-4). AA is the precursor for the 2-series prostanoids and the 4-series leukotrienes. DGLA competes with AA for COX and LOX, driving the synthesis of 1-series prostaglandins and inhibiting the synthesis of AA-derived eicosanoids. EPA and DHA also use COX and LOX to produce the 3-series prostanoids and 5-series leukotrienes. COX and LOX enzymes prefer AA over EPA as a substrate, and thus the synthesis of AA-derived eicosanoids is preferred over synthesis of EPA derived eicosanoids (Russo, 2009 and references therein). Nonetheless, high levels of EPA can decrease the production of AA-derived eicosanoids, thus impacting the actions of these mediators (Calder, 2008). AA-derived eicosanoids are generally pro-inflammatory and also play roles in platelet aggregation, immunity, smooth muscle contraction and renal function, while EPA derived eicosanoids are generally less potent, thus playing a role in reduction of inflammation (Muskiet *et al*., 2004; Calder and Yaqoob, 2009 and references therein). Indeed, it has been shown that n-3 PUFA are beneficial in improving the host immunity under a number of inflammatory conditions (Robinson *et al*., 1993; Grimble, 1998). Moreover, n-3 PUFA intake may improve resistance to infectious diseases by changing cytokine and/or eicosanoid synthesis (Anderson and Fritsche, 2002). Another family of lipid mediators are resolvins and protectins, which can be synthesized from AA, EPA and DHA (Figure 2-4), and which are potent anti-inflammatory, inflammation-resolving, and immunomodulatory substances. Finally, protectin D1, produced from DHA, seems to have a role in protecting tissues from excessive damage (Calder and Yaqoob, 2009 and references therein).

2.2.3 Other mechanisms of action of LCPUFAs

Increasing the n-3 LCPUFA concentration in cells and tissues in the body can alter the eicosanoid production as described above, and this is one of the mechanisms through which n-3 LCPUFA exert its effects. Another mechanism of action includes changes in the physical properties of cell membranes, like the fluidity, which influences the activity of membrane proteins, like receptors, thus assisting in correct hormone-receptor binding, and transporters, ion channels and signaling enzymes (Russo, 2009; Calder and Yaqoob, 2009). It is believed that n-3 LCPUFA decrease insulin resistance through the improvement of membrane fluidity, by enhancing the number of insulin receptors and increasing the affinity of insulin to its receptors (Das, 2006).

A third mechanism of action of n-3 LCPUFA includes regulation of transcription factors, altering gene expression. Peroxisome proliferator receptors (PPARs), liver X receptors (LXRs) and sterol regulatory element-binding protein 1c (SREBP-1c) are all nuclear receptors important in the regulation of fatty acid metabolism. N-3 PUFAs can increase the PPAR activity, which regulates several genes involved in lipid metabolism, like Δ5- and Δ6-desaturase. N-3 PUFAs can also decrease activation of SREBP-1c, thereby affecting transcription of lipogenic genes. Although n-6 and n-3 PUFAs are often interchangeable in regulating gene expression, n-3 PUFAs act as more potent ligands to nuclear receptors, thus altering transcription factor regulation (Russo, 2009 and references therein).

The pig has often been used as a model for humans. Smink *et al*. (2012) used young, growing pigs as a model for human infants. They stated that brain anatomy and morphology, the timing of the brain growth spurt, anatomy of the digestive system and many of the pathways of lipid metabolism in humans and pigs are very similar (Smink *et al*., 2012 and references therein). This means that results of pig trials with n-3 LCPUFA can be translated to humans, but also that results found in humans can be translated to pigs, which might be useful for the pork industry.

2.2.4 Dietary sources of n-3 LCPUFA

It is believed that both humans and livestock species evolved on a diet with a n-6 : n-3 PUFA ratio of 1:1 (Pike and Barlow, 2000), while the current Western diet is very high in n-6 PUFA and low in n-3 PUFA with a n-6 : n-3 PUFA ratio of 20-30:1, due to a decrease in fish consumption and an increase in consumption of n-6 PUFA rich foods, like meat and grains (Simopoulos, 1991). Cattle used to get their n-3 PUFA through fresh pasture, as grass is rich in ALA. However, modern livestock systems give little access to fresh pasture, instead feeding preserved forage with low PUFA concentrations, supplemented with fat derived from oilseeds rich in LA (Pike and Barlow, 2000). In the current Western pork production systems, pigs are fed either with a mixture of corn and soybean meal, or a mixture of wheat and barley, all of which are high in LA and low in ALA (Table 2-1). To increase the n-3 PUFA intake and improve the n-6 : n-3 PUFA ratio in livestock, diets can be supplemented with flaxseed or fish oil (Table 2-1). The

difference between flaxseed and fish oil is that flaxseed provides ALA, whereas fish oil provides EPA and DHA to the diet. As most reported health benefits by n-3 PUFAs are caused by EPA and DHA, feeding fish oil is believed to be more beneficial for health than feeding flaxseed. However, not all sources of fish oils are the same. Fish can be classified into lean fish that store lipid in the liver (like cod) or 'fatty' fish that store lipid in the flesh (like salmon and tuna) and they contain different amounts of fatty acids and different ratios of EPA to DHA (Calder and Yaqoob, 2009). Fish oil is obtained from fatty fish flesh or lean fish livers and is high in EPA and DHA, although the exact amounts and ratio of EPA to DHA depend on the source of the oil (i.e. different types of fish).

2.2.5 Uptake of fatty acids in the fetus

There is ample evidence that fatty acids in the maternal diet are taken up into the blood stream in mammals (Rooke *et al*., 2000; Geppert *et al*., 2007; Hanebutt *et al*., 2008; Brazle *et al*., 2009). The question then becomes if, and how, these fatty acids can reach the fetus during pregnancy. Fatty acid transfer across the placenta in humans has been extensively reviewed by Haggarty (2002), Cetin and Koletzko (2008) and Hanebutt *et al*. (2008). In the human there are only three layers between the maternal and fetal blood supply and, as a result, fatty acids can easily cross the placental membranes as free (non-esterified) fatty acids by diffusion. The placenta does not have the Δ5- and Δ6-desaturase enzymes, thus it cannot convert LA and ALA to LCPUFA. Also fetal desaturase activity is very limited, meaning that all LCPUFAs must be transported from the maternal blood to the fetus. Generally, AA and DHA concentrations are higher in fetal blood than maternal blood, indicating a preferential uptake of these fatty acids, especially DHA, by the placenta. A possible mechanism in preferential fatty acid uptake may involve fatty acid binding proteins and cytoplasmatic transport proteins that have been identified in the placenta. One placenta specific protein has higher affinities and binding capacities for AA and DHA compared with LA and oleic acids (Haggarty, 2002 and references therein). Haggarty *et al*. (1997) showed that selective uptake of fatty acids by the placenta from the blood was AA>DHA>ALA>LA. However, the selective transfer of fatty acids from the placenta to the fetus was different: DHA>ALA>LA>AA. The major difference in selective AA uptake by the placenta, but not to the fetus, shows that the placenta retains AA. It seems likely that the placenta has a requirement for AA to produce

eicosanoids and only when this requirement has been met, the remaining AA becomes available for transfer to the fetus (Haggarty, 2002 and references therein). The placenta may play a role in its own substrate supply through the action of placentally derived leptin. Leptin is a stimulator of lipolysis, which mobilizes fatty acid stores from maternal adipose tissues and increases the availability of free fatty acids. It is suggested that the placental leptin signal is related to the fetal growth rate, and thus that the placenta reacts to fetal demands (Haggarty, 2002).

Crawford *et al*. (1989; referenced by Leskanich and Noble, 1999) showed that AA and DHA levels in human umbilical cord plasma phospholipids were higher in placentae with a high vs. low weight, and they hypothesized that a deficiency of essential fatty acids and LCPUFA could lead to sub-optimal blood flow conditions in the growing placenta. However, other research has shown that fetuses that are small for gestational age (SGA) have an excess of circulating fetal lipid, meaning that "the availability of fatty acids within the fetal circulation, and hence the placental supply, is not the first limiting factor for fetal growth in SGA fetuses" (Haggarty, 2002 and references therein). On the other hand, research has showed that IUGR babies have lower fetal:maternal ratios for DHA and AA (meaning lower transplacental transfer) than babies with appropriate weight for gestational age, and that IUGR babies had lower enzymatic conversion from DPA to DHA, resulting in an impaired LCPUFA supply (Hanebutt *et al*., 2008 and references therein).

Compared to the information of placental transfer of fatty acids known in humans, much less is known for pigs. Overlap between pigs and humans may be limited, due to different placental structures (pigs: epitheliochorial, humans: hemochorial), and major differences in lipid stores at birth between humans (high) and pigs (very low). Some information on fatty acid transfer in the porcine placenta has been described by Leskanich and Noble (1999) and by Père (2003), who compared materno-fetal exchange between species. In humans, which have a permeable placenta, free fatty acids are readily transferred from the mother to the fetus, as described above, but this is not as efficient in pigs. Pigs have six layers between the maternal and fetal blood supply, and this makes the placenta less permeable. Free fatty acid levels in fetal blood are low and not correlated with the maternal levels. When labeled fatty acids were injected in sows, only trace amounts of these fatty acids crossed the placenta. Also in contrast with humans, it appears that the

porcine placenta does not store fatty acids (Leskanich and Noble, 1999). In humans, some placental lipoprotein lipase activity has been demonstrated, meaning that the placenta can use fatty acids from triglycerides to transfer to the fetus. Lipoprotein lipase activity has been reported to be high in the pig, and it seems to be only located on the maternal side of the placenta, so that reverse transfer of fatty acids from the fetal to the maternal circulation is prevented (Leskanich and Noble, 1999, and references therein).

2.2.6 Effect of maternal nutrition on fatty acid supply to offspring

Regardless of species and placental transfer mechanisms, fatty acids can only be transferred to the fetus if they are available in the blood supply of the mother. As blood fatty acid composition highly depends on nutrition, this means that the fatty acid composition of the maternal diet is the most important determinant of which fatty acids are delivered to the fetus. Indeed, it has been shown in pigs that supplementing maternal nutrition with corn oil or sunflower oil increased LA content of newborn piglets (Père, 2003 and references therein), while supplementing sows with tuna oil, rich in EPA and DHA, increased total n-3 PUFA, and particularly DHA, in umbilical cord, piglet plasma and piglet tissues (Rooke *et al*., 1999). To-date, only one paper has been published showing the direct effect of maternal LCPUFA supplementation (in the form of fish oil) on fatty acid composition of the endometrium, chorioallantois and fetus (Brazle *et al*., 2009). Fish oil supplementation increased DHA levels in the endometrium, chorioallantois and fetus around day 40 of gestation. Another trial, described in the same paper, showed that fish oil supplementation increased endometrial EPA, and EPA and DHA in extraembryonic tissues at day 11 to 19 of gestation, while it only increased DHA in embryos at day 19 of gestation (Brazle *et al*., 2009).

When sows were supplemented with n-3 LCPUFAs in gestation and lactation, n-3 LCPUFA levels were also higher in colostrum and milk (Taugbol *et al.*, 1993; Rooke *et al.*, 2000, 2001). Overall, therefore, piglets can benefit from n-3 LCPUFA supplementation to the sow in two ways; 1) prenatally, when developing embryos have access to DHA, and 2) postnatally, when litters consume colostrum and milk containing elevated concentrations of EPA and DHA. This is important information when trying to understand the effects of maternal n-3 LCPUFA supplementation to sows on piglet

growth performance, mortality rates and carcass quality. Specific results of n-3 LCPUFA supplementation to sows will be discussed in chapters 3, 5 and 6.

2.3 Summary

Many factors are involved in embryonic and fetal development. Among these, placental function plays a particularly critical role and inadequate placental function, linked to intrauterine crowding in early gestation, often leads to IUGR. IUC is believed to be the result of high ovulation rates, followed by fair to good early embryonic survival to day 30 of gestation. This results in more embryos in the uterus than can be supported to term. A wave of fetal loss then occurs between day 30 and 50 of gestation, resulting in normal litter sizes at term (between 9 and 16 total born). However, limitations in placental weight at day 30 due to IUC persist to day 50 and are then associated with decreased fetal weight and other characteristics of IUGR which persist to term. IUC has also been shown to decrease the number of muscle fibers, and this effect was independent of individual birth weight. Therefore, birth weight per se is not a factor for lower myofiber numbers; instead this is due to fetal programming of myogenesis during fetal development. This also means that intrauterine crowding can affect the postnatal growth performance of the entire litter, and not just the small pigs in that litter.

Knowing at birth which litters have lower growth potential may have important consequences for maximizing economic returns. This gives rise to the question how to identify litters at birth that underwent intrauterine crowding. Chapters 4 and 5 describe a method to classify litters as low, medium, or high birth weight. It was expected that the low birth weight litters described in this thesis would show the benchmarks of IUGR, measured as a lower placental weight and the occurrence of brain sparing. This was indeed the case. The question then becomes what the effects are of low litter average birth weight on post-natal growth potential and carcass quality. The repeatability of litter birth weight within sows was also investigated. Together, the results provide us with practical management tools to deal with low birth weight litters.

One of the proposed management tools to deal with sows with a predicted low birth weight phenotype is to use nutritional intervention with a supplement that could improve offspring survival and growth rates. N-3 LCPUFA supplementation to sows during

(parts) of gestation and lactation has been shown to improve growth rates in the offspring. Therefore, the other research described in this thesis deals with marine-oil based n-3 LCPUFA supplementation to gilts and sows during (parts of) gestation and lactation. Although the past 15 years has seen important advances in the understanding of the working of n-3 LCPUFA in the body, results of maternal n-3 LCPUFA supplementation on offspring development are still inconsistent. Moreover, specific effects of maternal n-3 LCPUFA supplementation on growth peformance and carcass quality of low birth weight litters have not been studied before. Therefore, Chapters 3, 5 and 6 describe a series of experiments to investigate effects of maternal marine-oil based n-3 LCPUFA supplementation on growth performance of all offspring, or specifically of low birth weight litters, and on subsequent reproductive performance of treated sows.

Table 2-1. N-6 and n-3 PUFA concentration in some ingredients used in pork production, as % of total fatty acids

	Total fat (% of weight)	n-6 PUFA		n-3 PUFA		n-6:n-3 ratio	Reference [a]
		18:2 LA	20:4 AA	18:3 ALA	20:5 EPA + 22:6 DHA		
Corn	3.9	50.5		0.9		50	1, 4
Wheat	2.0	56.3		3.7		15	1, 4
Soybean meal	1.0	51.5		7.3		7.1	1, 4
Barley	1.9	44.7		6.6		6.8	2, 4
Flaxseed	41.0	15.0		53.1		0.3	1, 5
Salmon	5.3-13.3	0.44	0.30	0.55	1.20	0.4	3, 6
Tuna	8.0	0.26	0.28	0.27	0.40	0.8	3, 7

[a] 1: Van Kempen, 2003, 2: Youssef *et al*., 2012, 3: Russo, 2009, 4: Lin *et al*., 2013, 5: Estwood and Beaulieu, 2010, 6: en.wikipedia.org/wiki/tuna, visited 25th of March 2013, 7: www.seafoodhealthfacts.org/seafood_choices/salmon.php, visited 25th of March 2013

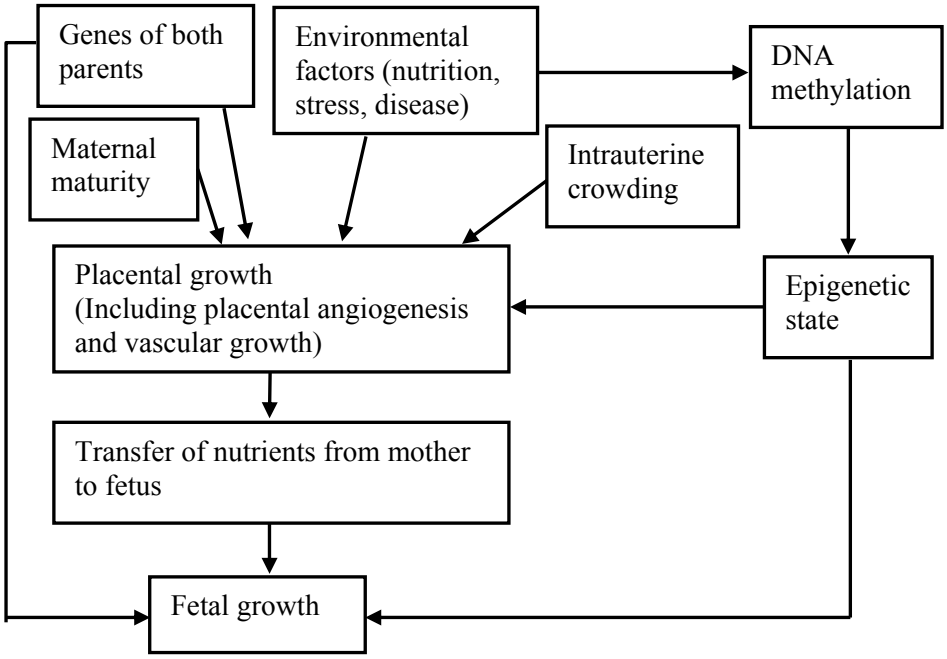

Figure 2-1. Factors influencing mammalian fetal growth (adapted from Wu *et al.,* 2006)

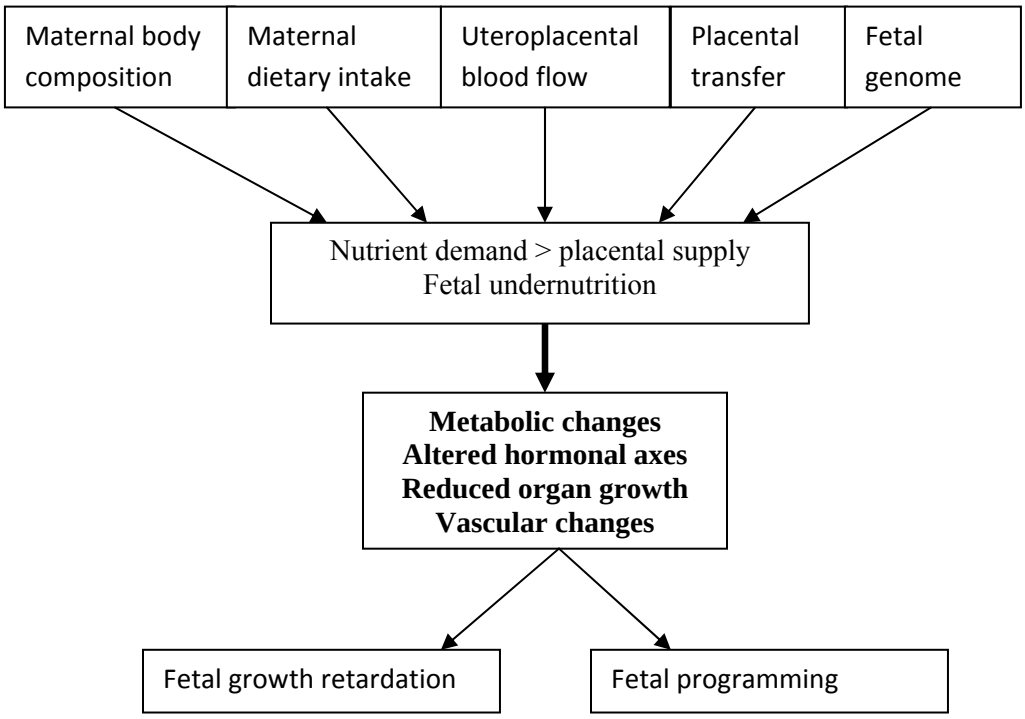

Figure 2-2. How fetal undernutrition may lead to long term changes in physiology which predispose to cardiovascular and metabolic disease (Phillips, 2006)

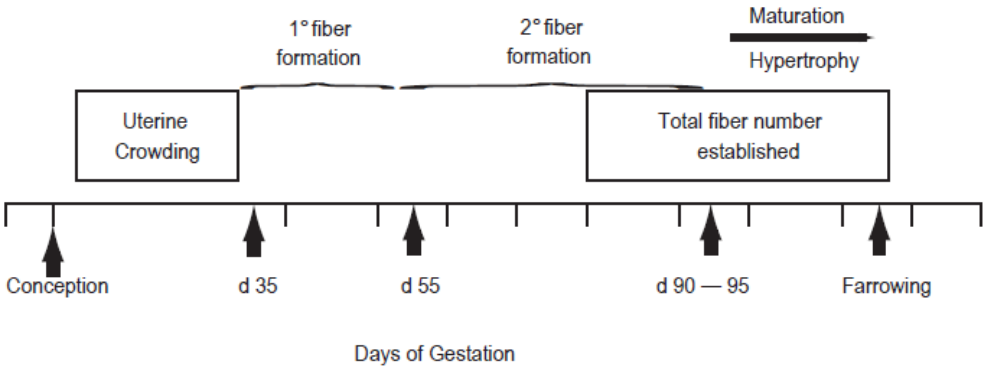

Figure 2-3. Schematic representation of the time-course of muscle fibre development in the pig, indicating a critical window in early pregnancy when crowding effects limit placental development and set in place detrimental effects on fetal development and lifetime growth performance (Foxcroft *et al*., 2006).

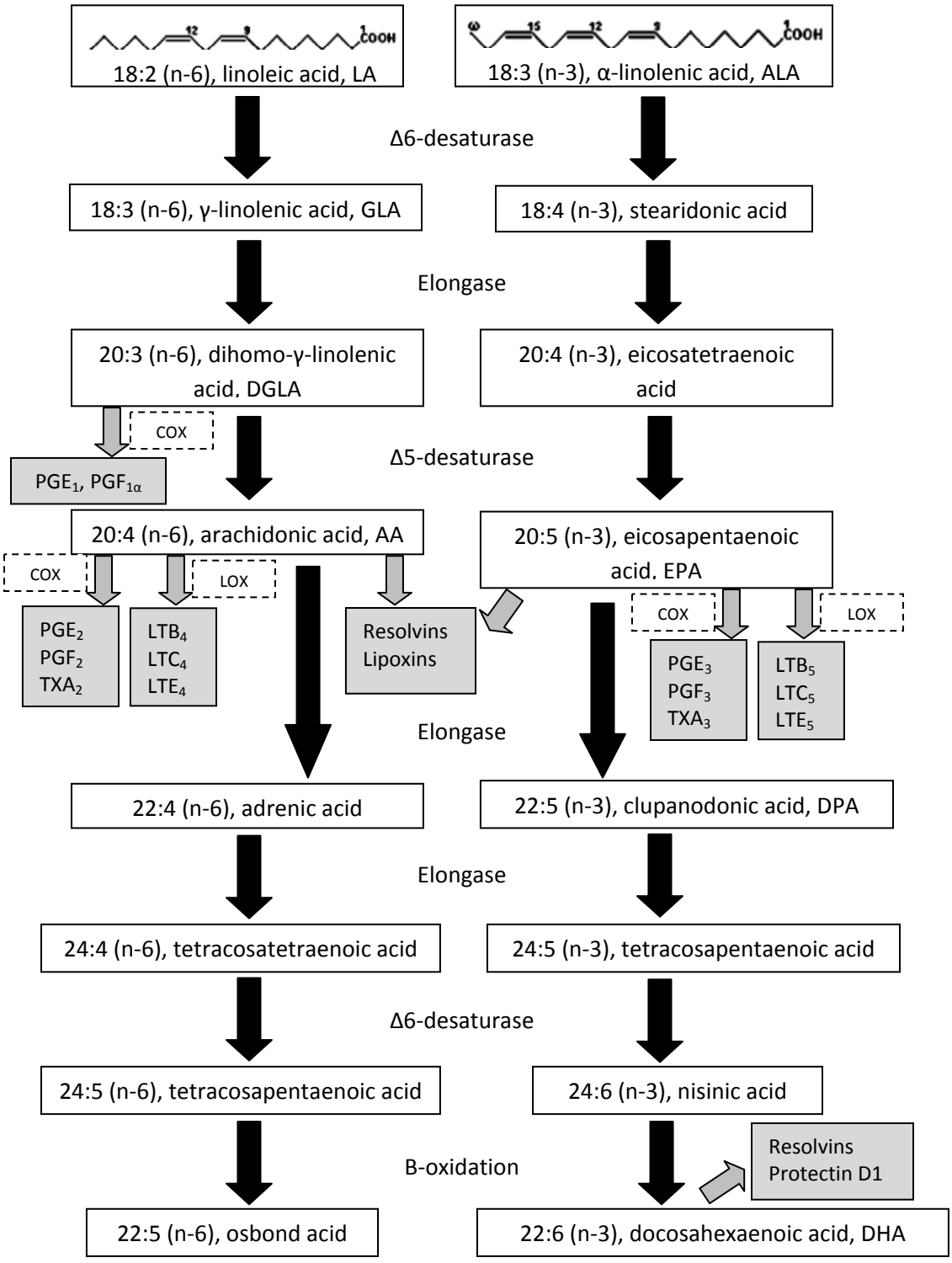

Figure 2-4. N-3 and n-6 LCPUFA biosynthesis. Mammals convert LA and ALA to LCPUFA using a series of desaturation and elongation reactions. The synthesis of eicosanoids by COX and LOX enzymes, as well as the formation of resolvins and protectins is also shown. (adapted from Russo, 2009)

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Chapter 3: Responses to marine-oil based n-3 LCPUFA supplementation of gestating gilts, and lactating and weaned sows [1]

3.1 Introduction

Studies in sows have shown benefits of n-3 long chain polyunsaturated fatty acid (**LCPUFA,** 20 or more C atoms) supplementation on post-natal growth (Rooke *et al.*, 2000 and 2001b; Mateo *et al.*, 2009) and pre-weaning mortality of the litter (Rooke *et al.*, 2001a). Others report an increase in live pigs born in gilts (Edwards and Pike, 1997; Spencer *et al.*, 2004) and sows (Webel *et al.*, 2003 and 2004; Smits *et al.*, 2011) when supplementing with n-3 LCPUFA at various stages of gestation, lactation and/or during rebreeding, and Webel *et al.* (2004) hypothesized that the observed increased litter size was due to improved embryonic survival at day 30 of gestation, rather than differences in ovulation rate. However, others report no effects of n-3 LCPUFA supplementation to sows on litter size at birth (Rooke *et al.*, 2001a; Estienne *et al.*, 2006) or on embryonic survival (Estienne *et al.*, 2006). Even when only comparing trials that used a protected source of n-3 LCPUFA and/or that measured n-3 LCPUFA uptake in the body, results are inconsistent. It is, therefore, important to further define situations in which positive effects of n-3 LCPUFA supplementation on reproductive performance and litter characteristics might be expected.

In the present study, which used a marine-oil based source of n-3 LCPUFA stabilized against auto-oxidation, two hypotheses were tested: 1) that feeding a supplement rich in stabilized n-3 LCPUFAs to gilts in established gestation would improve the growth performance of their litters; 2) that continued feeding of the supplement during lactation and after weaning would offset the expected negative effects of lactational catabolism induced using an established experimental model involving feed restriction of primiparous sows in late lactation (Patterson *et al.*, 2011). The fatty acid composition of sow serum, recovered embryos and corpora lutea (**CL**), was used to confirm effective transfer of n-3 LCPUFAs to potential target tissues.

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3.2 Material and Methods

3.2.1 Animals and treatments

This study was performed according to Canadian Council on Animal Care guidelines and with approval of the Faculty Animal Care and Use Committee – Livestock, University of Alberta (Protocol 2006-11C). Primiparous Large White x Landrace terminal line sows (n=117; Genex Hybrid; Hypor, Regina, SK, Canada) used between August 2008 and May 2009 were managed according to approved protocols at the Swine Research and Technology Centre (**SRTC**), University of Alberta. Herd protocols target gilts to be bred at least at second oestrus and within a weight range of 135 – 150 kg (mean breeding weight for this study =139.2 ± 9.8 kg). At day 60 of gestation, gilts were pair-matched within breeding group by body weight, and when possible by litter of origin, and within a pair (n=54) randomly allocated to be fed either standard SRTC gestation and lactation diets (**CON**; Table 3-1), or the same diets top-dressed daily with 84 grams of a marine-oil based n-3 LCPUFA supplement (**mLCPUFA**; Table 3-1), according to company recommendations. The n-3 LCPUFA product used (Sow Fat Pack 10, JBS United Inc, Sheridan, IN, USA) was a marine-oil based supplement rich in eicosapentaenoic acid (**EPA**) and docosahexaenoic acid (**DHA**) which was stabilized to prevent auto-oxidation. Two samples from each of the two batches of Sow Fat Pack 10 used in the trial were taken at the end of each batch and sent to the University of Missouri (Columbia, MO, USA) for fatty acid analysis. The fatty acid profile of both batches is shown in Table 3-2. The marine-oil based n-3 LCPUFA supplement was fed to gilts from day 60 of gestation, through a 21-day lactation, and until euthanasia of sows at day 30 of their second gestation. Nine gilts not allocated to a pair due to uneven numbers of gilts in some breeding groups were considered an 'incomplete pair' in the analysis.

3.2.2 Management during gestation

Bred gilts were housed in individual stalls until confirmed pregnant at approximately day 30 of their first gestation, and then as breed groups in "free access" gestation pens, consisting of 12 individual walk in/walk out stalls accessed from a common lounging area. Although sow pairs (treatments) within a breed group were located in the same gestation pen, access to the common area was alternated between the two treatment groups by locking gilts of the same treatment into individual stalls for alternating 7-day periods to facilitate hand-feeding of the marine-oil based LCPUFA supplement. Sows were offered between 2.0 and 2.8 kg/day of

the standard SRTC gestation diet (Table 3-1) during gestation, based on body weight and backfat thickness at breeding (see Matrix in Table 3-3). A standard 2 kg amount of feed was automatically dropped into individual feeders and gilts were then hand-fed the balance of their allowance.

3.2.3 Management during lactation and after weaning

Management of sows and litters in lactation, and sow management after weaning, followed the protocols described for the Restrict sows in the study of Patterson *et al.* (2011) with some modifications. In summary, to facilitate farrowing room management and to standardize lactation length, sows that did not show signs of farrowing by day 113 of gestation (calculated from the first AI date) were induced to farrow using prostaglandin F2α injections (Estrumate, Intervet Canada Corp., Kirkland, QC). Within 48h after farrowing, litter size was standardized to 10-13 piglets per sow by cross-fostering within treatment groups where possible. For 9 litters, cross-fostering between treatments occurred and was recorded. In the farrowing rooms, sows were offered the standard SRTC gilt lactation diet (Table 3-1) at 3.0 kg/day until the day of parturition. Sows were fed at the discretion of the farrowing technician on the day of parturition. From day 1 of lactation to 7 days before weaning (Wn-7), sows were fed a "step-up" regimen to appetite with no maximum daily feed allowance being implemented. From Wn-7 until weaning, sows were fed 60% of their expected feed intake, calculated using feed intake in the last 3 days before restriction (Wn-8-10). Treated sows had to fully consume the marine-oil based LCPUFA top-dressed feed first offered each morning before further fresh feed was distributed. Litters were weaned on day 21.4 ± 1.5 of lactation.

Sow management after weaning until breeding was as described previously (Patterson et al., 2011), but mLCPUFA sows were again offered the marine-oil based LCPUFA supplement with a limited amount of feed each morning and required to consume this feed before further feed was offered to appetite. During their second gestation until slaughter, sows were housed in individual stalls and fed the standard gestation diet between 2.2 and 3.0 kg/day, again taking into account body weight and backfat thickness at breeding (see Matrix in Table 3-3). Sows were provided with front stall exposure to a rotation of mature boars once a day for 30 minutes during the first 3 days after weaning. From day 4 after weaning, sows were heat checked as previously described (Patterson *et al.*, 2011). From October to mid-December sows were bred by artificial insemination

using fresh, pooled semen (3 x 10^9 spermatozoa/dose) collected on-site from proven terminal-line, Duroc boars (Hypor Magnus, Hypor, Regina, SK, Canada). Each pool contained equal numbers of sperm from at least 3 different boars. From mid-December until the end of the trial in April, sows were switched to single-sire inseminations (2 x 10^9 spermatozoa/dose) using semen from one of two proven terminal-line boars, ensuring that the distribution of boars was equal between treatments. All semen was used within 3 days of collection and extended in BTS medium (Minitube of America, Inc, Verona, WI, USA).

At day 30.3 ± 0.8 of gestation (counting the time of the last AI as day 1) pregnant sows were euthanized on-site by qualified staff using approved necropsy procedures and their reproductive tracts were recovered for dissection.

3.2.4 Litter management after weaning

After weaning, piglets were moved to an assigned nursery room and penned by litter if possible. However, if there were more litters than pens, some litters were divided over several pens within treatment. Pens were 147.3 cm by 223.5 cm, with fully slatted plastic flooring. Nursery pigs were fed with a phase-feeding program according to SRTC nursery guidelines (See Table 3-4) and water was freely available at all times.

3.2.5 Calculations of energy input and output in lactation

Metabolic state of lactating sows was calculated as described by Bergsma *et al.* (2009) and characterized using the approach described by Patterson *et al.* (2011). In summary, energy from feed intake was calculated using the 'ME swine' given for the diet multiplied by the amount of feed disappearance. Energy input consisted of energy from total feed intake and body tissue mobilisation of the sow, minus energy needed for maintenance. Energy output was calculated as energy needed for piglet growth and maintenance. As described by Patterson *et al*. (2011) a subpopulation of sows were identified that mobilised higher amounts of body tissues to support litter weight gain and were considered *at risk* of inducing negative effects on embryonic development of the subsequent litter.

3.2.6 Progesterone assay

Heparinised blood samples collected 60-72 hours after expected time of ovulation from each sow by jugular venipuncture during nose-snare restraint, were centrifuged (GPKR centrifuge: Beckman, Fullerton, CA, USA) at 569 × *g* and 4°C for 15 minutes, and plasma harvested and frozen at -20°C until assayed in triplicate using the method of Mao *et al.* (1998). The volume of sample taken to assay was 0.1 ml of a ten-fold dilution of plasma in kit buffer. Assay sensitivity for the three assays completed, defined as 85.66 %, 91.29 % and 89.55 % of total bound, respectively, averaged 0.0086 ng/tube, equivalent to 0.86 ng/ml for the diluted samples. None of the diluted samples fell below assay sensitivity. The intra-assay CVs were 3.90 %, 3.57 % and 1.09 %, respectively for the three assays and the inter-assay CV for a standard internal control plasma sample run in each assay was 6.04 %. A serially diluted plasma pool showed parallel inhibition to the standard curve.

3.2.7 Fatty acid composition analysis in serum and tissues

Additional blood samples were taken by jugular venepuncture at day 60 and 110 of first gestation and at day 30 of second gestation (euthanasia) to measure fatty acid composition of blood. Blood samples were collected into non-heparinised vacutainer tubes (BD, Fisher Scientific, Ottawa, ON, Canada), centrifuged at 569 × *g* and 4°C for 15 minutes, and serum was harvested and frozen at -20°C until further analysis. Samples from ten sow pairs were randomly chosen for analysis of fatty acid composition in serum at day 110 of first gestation and day 30 of second gestation, and in luteal and embryonic tissues collected at day 30 of gestation.

All chemicals used were obtained from Fisher Scientific (Ottawa, ON, Canada), unless otherwise stated. Lipids were extracted by mixing 2 ml of each serum sample with 10 ml methanol and 20 ml chloroform. After shaking, the samples were allowed to sit for one hour and then 4 ml 0.88% (w/v) NaCl was added and the samples were centrifuged at 327 × *g* for 5 minutes to separate layers. Ten ml of the chloroform layer was transferred to another tube and evaporated to dryness under nitrogen. The extracted lipids were dissolved in a recorded volume of between 100 and 150 µl of chloroform and 50 µl was transferred to a different tube. Transesterification of fatty acids was accomplished by adding 2 ml of methanolic HCl (Sigma-Aldrich Inc., St. Louis, MO, USA) and the samples were placed in a water bath for 2 hours at 60°C with frequent vortexing. After

cooling, 2 ml water, 3 ml hexane and 100 µl of an internal standard (containing 1 mg C17:0 per ml of hexane) were added and after shaking, samples were centrifuged at 581 × *g* for 5 minutes to separate layers. The majority of the upper hexane layer was transferred to another tube containing a pinch of anhydrous sodium sulphate (Sigma-Aldrich Inc., St. Louis, MO, USA). Samples were centrifuged again at 581 × *g* for 2 minutes and approximately 1 ml was transferred to chromatography vials.

Four embryos of average size were chosen for analysis of each selected sow (2 embryos from the middle of the left uterine horn and 2 embryos from the middle of the right uterine horn) and all frozen CL (2 from the left and 2 from the right ovary) of each selected sow were used for tissue analysis. Individual embryos and CL were ground under liquid nitrogen with a mortar and pestle, weighed, lyophilized and weighed again. Embryonic and luteal samples were then pooled within sow, and triplicates of 25 mg per sample were directly methylated using 2 ml of methanolic HCl as described above. The samples were then diluted in hexane by a factor 3.3 for embryos and a factor 4 for CL before gas chromatography.

Fatty acids were analyzed by a gas chromatograph (model Varian 3400; Varian Inc, Mississauga, ON) and used a flame ionization detector. It was equipped with a Varian 8100 auto sampler and used a SP-2560 fused silica capillary column (100 m x 0.25 mm i.d. x 0.2 µm film thickness; Supelco Inc., Bellefonte, PA). Hydrogen was the carrier gas. A cool on-column injection was used. The injector program started at 50 ºC and was immediately increased to 230 ºC at 150 ºC/min and held for 83 minutes. The column was operated at 45 ºC for 4 min, then temperature-programmed at 13 ºC/min to 175 ºC, held there for 27 min, programmed at 4 ºC/min to 215 ºC, and finally held there for 35 min; total run time was 86 min. The putative 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). Data was integrated using Galaxie Chromatography Data System (Varian Inc., Mississauga, ON). 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. To calculate the actual amount of each fatty acid (µg/ml serum or µg/g embryo) an internal standard (C17:0) was used.

3.2.8 Statistical analysis

All variables were tested for normality prior to analyses, using the univariate procedure in SAS. Also the homogeneity of variance of the residuals was tested for each variable, using the Bartlett and Levine's tests in SAS. When variables were not normally distributed, they were only transformed if the variance of residuals was not homogeneous and if it improved the fit of the model. None of the variables needed transformation on this basis.

Data were analyzed using the MIXED procedure of SAS (SAS Institute, Cary, NC, USA) as a randomized incomplete block design, with blocks based on sow pairs. If one sow of a pair was taken off trial, the other sow of the pair stayed on trial and was considered an incomplete block. The model included treatment (CON or LCPUFA) as a fixed effect and pair as a random effect. Sow was used as the experimental unit for all parameters tested, including determining treatment effects on litter growth and on reproductive traits at day 30 of gestation, and all individual measurements of piglets, embryos and CL were averaged within a litter (sow) before statistical analysis. Repeated measures analysis was used for sow weight, backfat, and for piglet body weight. An appropriate covariance structure was selected by comparing the goodness-of-fit measures of different structures. The Kenwardroger approximation was used for the denominator degrees of freedom. Categorical data like breeding rate, pregnancy rate and pre-weaning mortality were analyzed separately using the generalized logit function (proc CATMOD in SAS).

To account for small differences in energy input due to the additional 84 g/day of supplement provided to mLCPUFA sows during lactation, energy input from feed in the last 7 days of lactation was used as covariate for analyzing treatment effects on piglet body weight.

Associations between fatty acids in serum versus embryos and CL were measured using correlation analysis.

Relationships between total energy input derived from body tissue mobilization and embryo weight and average litter growth rate were analyzed using the 'quadrant' analytical approach described by Van den Brand *et al*. (2000) and Vinsky *et al*. (2006). A simple linear regression equation was used to find positive linear relationships between day of gestation at euthanasia and average embryo weight per litter for CON (-8.42 + 0.35 gestation day; $R=0.80$; $P<0.001$) and mLCPUFA (-6.11 + 0.27 gestation day; $R=0.76$; $P<0.001$). Therefore, day of gestation was used as a covariate for embryonic weight, embryo crown-rump length and allanto-chorionic fluid volume. Moreover,

average litter embryo weights were adjusted for the day of gestation. Based on these findings, a subpopulation of sows was identified that mobilized higher levels of body tissue stores (40 MJ ME/day) and was classified as 'at risk' of inducing negative effects on embryonic development of the subsequent litter.

Data in the text are given as least square means ± S.E.M., unless otherwise stated, and data in the figures as means. Probability values < 0.05 were considered significant and values < 0.10 were used to describe trends.

3.3 Results

Of the 117 sows allocated to treatment, data from nine were removed from all analyses due to cross-fostering of litters between treatments (See Discussion below). Of the 108 sows remaining, 104 sows (52 CON and 52 mLCPUFA) farrowed normally (one CON and one mLCPUFA sow aborted, one mLCPUFA sow was taken off trial due to lameness and one mLCPUFA sow farrowed in the gestation room). During lactation, four sows (all mLCPUFA) were taken off trial, either due to low feed intake (2 sows), or health reasons (2 sows). At weaning, four sows (all CON) were taken off trial due to demands of other research projects. One CON and two mLCPUFA sows did not return to oestrus after weaning and five sows (2 CON and 3 mLCPUFA) were not pregnant. This resulted in 45 CON and 43 mLCPUFA sows being available for study at day 30 of second gestation.

3.3.1 Sow feed intake and sow measurements in lactation

Feed intake was similar between treatments during the first 2 weeks of lactation (Figure 3-1a). Although feed intake during the 3 days before restriction was not different for CON and mLCPUFA sows (6.53 ± 0.15 vs. 6.76 ±0.15 kg/day, respectively), mLCPUFA sows had higher ($P<0.05$) feed intake (3.63 ± 0.04 kg/day) than CON sows (3.51 ± 0.04 kg/day) during the restriction period (Figure 3-1a). This was followed by a lower ($P<0.05$) feed intake in mLCPUFA (4.61 ± 0.14 kg/day) than in CON (5.04 ± 0.14 kg/day) sows in the 6 days after weaning (Figure 3-1b).

Sow backfat thickness was not different between treatments at any time during the trial (Table 3-5). There was an interaction between sow body weight and time ($P=0.05$). MLCPUFA sows tended to have higher body weight at day 1 of lactation than CON sows (181 vs. 179 kg, resp., $P=0.08$), whereas there was no difference in body weight between

treatments at other time points during the trial (Table 3-5). Weight loss during lactation was on average 18.9 ± 6.3 kg (mean ± st.dev.) and was similar between treatments.

3.3.2 Litter measurements

The total number of pigs born, pigs born alive, stillborn and mummified per litter, and average litter birth weights, were similar between treatments (Table 3-6). Pre-weaning mortality was higher (P=0.05) in mLCPUFA (13.2%) than CON litters (9.6%). For both treatments, the biggest contribution to pre-weaning mortality was piglets crushed by the sow (45.0% for CON and 48.1% for mLCPUFA litters, respectively). There was a tendency (P=0.06) for litters from mLCPUFA sows to have improved overall growth performance (average piglet weight) to the end of the nursery stage (34 days after weaning) compared to litters from CON sows (Figure 3-2).

3.3.3 Sow reproductive characteristics

The weaning-to-oestrus interval, breeding rate, pregnancy rate and day of gestation at euthanasia were similar between groups (Table 3-7). Treatment did not affect ovulation rate, number of live embryos, embryonic survival, embryonic weight, embryo crown-rump length or allanto-chorionic fluid volume (Table 3-7). However, average CL weight was higher (P<0.05) in mLCPUFA than CON sows (Table 3-7).

Progesterone concentrations in plasma 60-72 hours after calculated time of ovulation (9.94 ± 0.62 mg/l for CON (n=36) and 9.17 ± 0.64 mg/l for mLCPUFA (n=33)) were not different (P=0.36).

3.3.4 Fatty acid concentration in sow serum, embryos and CL

Concentrations of EPA and DHA in serum were higher, and concentrations of the n-6 LCPUFA arachidonic acid (**AA**) were lower, in mLCPUFA than CON sows at day 110 of first gestation and at euthanasia at day 30 of the second gestation (Table 3-8 and Table 3-9). In embryos, only DHA was higher in mLCPUFA compared to CON sows, while in CL both EPA and DHA were higher in mLCPUFA than CON sows (Table 3-8 and Table 3-9). AA tended to be lower ((P=0.08) in embryos and was significantly lower (P=0.01) in CL from mLCPUFA compared to CON sows. Total n-3 LCPUFA increased in mLCPUFA compared to CON sows in serum at day 110 and euthanasia, and in embryos

and CL, causing the n6:n3 ratio to decrease for mLCPUFA vs. CON sows in all samples (Table 3-8 and Table 3-9).

Concentrations of both DHA and AA in embryos and serum at euthanasia were correlated (DHA, R=0.66, P<0.01; AA, R= 0.65, P<0.01), whereas only a trend for a correlation between DHA concentrations in CL and in serum at euthanasia was established (R=0.39, P=0.10).

3.3.5 Sow energy input and output calculations in lactation

For all energy input and output calculations, only sows with all data available were used (CON: n=40 and mLCPUFA: n=35). Energy input from feed was similar in the first 2 weeks of lactation, while energy input from tissue mobilization tended to be higher in CON than mLCPUFA sows (P=0.10) and energy used for maintenance was higher in mLCPUFA than CON sows (P<0.001; Table 3-10). Nonetheless, total estimated energy input to the litter for CON and mLCPUFA sows was not different between treatments. Estimated energy requirements for litter maintenance and growth, and hence total energy outputs to the litter for CON and mLCPUFA sows, were not different (Table 3-10). Lactation efficiency of CON and mLCPUFA sows, defined as the energy efficiency of sows during lactation (output x 100 / input; Bergsma *et al.*, 2009), was also not different in the first 2 weeks of lactation. During the last 7 days of lactation, energy input from feed tended to be higher in mLCPUFA sows compared to CON sows (P=0.09; Table 3-10), energy input from tissue mobilization was not different, and energy used for maintenance was higher (P<0.01) in mLCPUFA than CON sows. Estimated total energy input to the litter and energy output for litter maintenance, growth and total output, did not differ between treatments. Lactation efficiency during the last week of lactation was similar between treatments and was higher than for the first 2 weeks of lactation (Table 3-10).

The relationship between energy input from body tissue mobilisation during the last week of lactation and embryo weight is shown in Figure 3-3a: Embryos tended to be heavier (P=0.09) in CON than mLCPUFA sows in this subset. Figure 3-3b shows the relationship between energy input from sow body tissue mobilisation during the last week of lactation and ADG of the litter. No effect of treatment on litter weight weaned was seen in this subset of sows.

3.4 Discussion

Auto-oxidation of fish oil in prepared feeds can be a problem if precautions are not taken (Fritsche and Johnston, 1988). The validity of data from studies not reporting stability of n-3 LCPUFAs and evidence for availability and/or transfer must be called to question, as it is likely that the level of n-3 LCPUFA received by the sows in those experiments was not similar to the analyzed n-3 LCPUFA levels in the feed at the beginning of the experiment. The marine-oil product used in the current trial was processed to stabilize the n-3 LCPUFAs, and EPA and DHA levels were monitored and analyzed throughout the trial (see Table 3-2).

Evidence that n-3 LCPUFAs in the sow's diet are taken up into the blood stream (Fritsche *et al.,* 1993; Rooke *et al.*, 2000; Brazle *et al.*, 2009) was confirmed by a 10-fold increase in serum EPA and DHA of marine-oil based n-3 LCPUFA supplemented sows in the present study, and as also reported by Rooke *et al*. (2000), was associated with a decrease in serum n-6 LCPUFA AA concentrations, while saturated fatty acids (SFA) and mono-unsaturated fatty acids (MUFA) were not different in blood when calculated as actual concentrations. SFA and MUFA were also similar between treatments in embryos and CL. An increase in only DHA in embryos at day 30 of gestation in the present study is consistent with the results of Brazle *et al.* (2009). This confirms that either DHA is taken up through the placenta into the embryo during early pregnancy or that more EPA was taken up by the embryo and then converted to DHA. As tissues of pigs from n-3 LCPUFA supplemented sows also showed higher EPA and DHA levels at birth than pigs from Control sows (Rooke *et al*., 2001b and 2001c), it is likely that n-3 LCPUFA uptake continues throughout gestation, although Amusquivar *et al.* (2010) showed that n-3 LCPUFA levels were increased in sow milk and pig plasma when sows were only supplemented with n-3 LCPUFA during the first half of gestation (day 1-60), suggesting that n-3 LCPUFAs can be stored in maternal adipose tissue and mobilized during milk production. When sows were supplemented with n-3 LCPUFAs in gestation and lactation, n-3 LCPUFA levels were also higher in colostrum and milk (Taugbol *et al.*, 1993; Rooke *et al.*, 2000, 2001a). Overall, therefore, piglets can benefit from n-3 LCPUFA supplementation to the sow in two ways; 1) prenatally, when developing embryos have access to DHA, and 2) postnatally, when litters consume colostrum and milk containing elevated concentrations of EPA and DHA. The growth of pigs that receive n-3 LCPUFAs only in gestation (through the placenta), or lactation (through

milk), or both might, therefore, differ, although Gabler *et al.* (2009) reported increased ex vivo active glucose uptake by the proximal jejunum of 21-day old pigs from sows fed n-3 LCPUFA supplements during gestation, during lactation, or both. Moreover, Rooke *et al.* (2001c) reported that when sows were only fed with n-3 LCPUFAs from salmon oil during the last part of gestation, piglets still grew faster after birth. On this basis, it was decided to exclude all data from the nine sows and litters in the present study in which cross-fostering between treatments occurred.

Because the marine-oil based n-3 LCPUFA supplement was provided in addition to the standard gestation and lactation diets at a rate of 84 g/d, starting at day 60 of gestation, treated sows received 4.5 kg more total feed than control sows over the last 54 days of gestation, which corresponds to a total of 35.7 MJ of energy intake. Although it is believed that energy provided through lipids is mainly transferred to the mammary glands (Van den Brand *et al.*, 2000; Quiniou *et al.*, 2008), it seems in the current trial that this extra energy was also used to support an increase in sow body weight, leading to a tendency for a higher body weight at farrowing in treated sows. This is in contrast with findings from Heo *et al.* (2008), who fed diets with 3 different energy levels to sows in gestation and lactation, and did not see any effect on sow body weight or backfat during gestation. The difference in energy levels between the highest and lowest treatments was 0.57 MJ/d in the study of Heo *et al.* (2008), whereas the energy difference in the current trial between control and treated sows was 0.66 MJ/d (~ 3% of gestation energy intake) This may have been enough to trigger a higher body weight at farrowing. However, as compared to Heo et al. (2008), gilts in the current trial were pair-matched by body weight and litter of origin at d60 of gestation. Pairs were included in the statistical analysis as random effect, which increases the power of the analysis and many more gilts were used in this trial (n=108) than by Heo *et al*. (2008; n=36). These two factors are thought to explain why smaller differences in energy intake and body weight could be picked up in the present study.

During the first two weeks of lactation sows were fed using a “to appetite” step-up regimen, and feed and energy intakes were similar between treatments during this period. During the last 7 days of lactation, energy intake was higher for treated than control sows. This was partly due to the 84 g/day of supplement added to the standard feed allowance and partly to a (non-significant) higher feed intake (202 g/day) of the standard

lactation feed in treated sows during the 3-day period before restriction (which was then used to calculate feed allowance during the restriction period). Because of the possible confounding effects of increased energy intake on piglet growth of treated sows, energy intake during the last 7 days of restriction was used as a covariate in analyzing possible effects of marine-oil based LCPUFA treatment on piglet weight and ADG.

Piglet birth weight was similar between Control and sows fed marine-oil based n-3 LCPUFA from day 60 of gestation onwards, consistent with the results of Mateo *et al*. (2009). Body weight from birth until the end of the nursery period (34 days after weaning) tended to be higher in litters from marine-oil based n-3 LCPUFA fed sows in the present study, again consistent with earlier studies (Rooke *et al.* 2000 and 2001b; Mateo *et al.* 2009). As Taugbol *et al.* (1993) and Fritsche *et al.* (1993) did not find an effect on weaning weight when n-3 LCPUFA supplementation started at day 107 of gestation, and Smits *et al.* (2011) did not find an effect on piglet growth and weaning weight when feeding n-3 LCPUFAs from 8 days before farrowing, longer periods of supplementation may be needed in gestation to produce positive effects on litter weaning weight.

Several mechanisms have been suggested as mediating effects of n-3 LCPUFAs on growth performance and survival, either through direct incorporation of n-3 LCPUFAs in tissues of offspring, or through expression of lipogenic enzymes in those tissues, which affects the biosynthesis of LCPUFAs from dietary precursors. Indeed, Missotten *et al.* (2009) showed that expression of Δ5- and Δ6-desaturase was tissue-specific, which the authors suggested was, at least partially, the reason for differences seen in n-3 LCPUFA levels between tissues.

A mechanism through which n-3 LCPUFAs can influence growth performance and survival is by improving the immune system. IgG in colostrum is the main source of antibodies that boost the neonatal pigs passive immune system and colostral IgG concentrations were greater in sows fed a n-3 LCPUFA rich diet (Mateo *et al.* 2009) and fatty acids influenced the expression of immune related genes (Jump and Clarke, 1999; Kitajka *et al.*, 2004). As discussed earlier, LCPUFAs also influence post-natal growth by improving gut development and integrity (Gabler *et al.* 2007 and 2009) and feeding n-3 LCPUFA to sows from day 109 of gestation until weaning decreased E.coli numbers in

the caecum and increased villous height in the ileum (Leonard *et al.* 2011), suggesting an improved gastrointestinal environment. Finally, n-3 LCPUFA can influence post-natal growth rate and survival through a change in piglet behaviour. DHA is important for brain development (Innis, 2007) and in central dopamine metabolism (Ng and Innis, 2003), which in turn affects feeding behaviour (McEntee and Crook, 1991). Rooke *et al.* (2001b) showed that piglets of sows supplemented with n-3 LCPUFA from day 92 of gestation to term tended to contact the udder more quickly and grasped a teat quicker than piglets from control sows. Furthermore Ng and Innis (2003) showed that pigs fed high n-3 PUFA levels had increased activity and decreased fear/anxiety when placed in an elevated plus maze. In another study, Rooke *et al.,* (2001a) showed that inclusion of salmon oil (rich in n-3 LCPUFA) in the sow's diet decreased pre-weaning mortality from 11.7% to 10.2%, mainly due to a reduction in piglets crushed by the sow. This is in contrast with findings from the present study, where pre-weaning mortality was higher in marine-oil based n-3 LCPUFA supplemented sows than control sows. Although not in itself significantly different between groups, the higher pre-weaning mortality in marine-oil based n-3 LCPUFA supplemented sows was mainly accounted for by a higher number of piglets crushed by the sow. It is not clear why this happened. However, Rooke et al. (2000) also found higher mortality rates in litters from n-3 LCPUFA fed sows, which they suggested was due to induction of the n-3 LCPUFA supplemented sows. Indeed, gestation length has been shown to increase due to n-3 LCPUFA supplementation (Rooke et al., 2001a) and induction at a standard time may have resulted in piglets being born more prematurely in respect of the natural gestation length of the sow and thus be less well prepared for birth. Indeed, most sows in the current trial were induced. However, one would expect to find lower birth weights if piglets were born prematurely, and this was not the case both in the current trial and in Rooke et al. (2000). In conclusion, interpretation of effects of marine-oil based n-3 LCPUFA treatment on survivability in this study may be difficult due to confounding effects of gestation length and the use of induced farrowing.

At day 30 of the second gestation, 88 sows (31 complete sow pairs and 26 incomplete pairs) were still on trial, which provided sufficient statistical power to determine potential effects of marine-oil based n-3 LCPUFA supplementation on reproductive performance after weaning, using an established feed restriction model developed to mimic the catabolic state frequently reported in primiparous sows during lactation, resulting in the

'second parity dip' (smaller number of piglets born and/or lower average litter birth weight in the second parity) and which resulted in reduced litter weaning weights and embryonic weights at day 30 of the following gestation in rebred sows (Patterson *et al.* 2011). The concept behind the present study was that this feed restriction model would provide a standard background challenge against which to determine beneficial effects of marine-oil based n-3 LCPUFA supplementation on both lactation performance and subsequent reproduction. However, even when comparing the reproductive performance of Control animals in the current trial with the equivalent Restricted group in the study of Patterson *et al.* (2011), higher breeding and pregnancy rates, and a similar ovulation rate but a higher number of live embryos due to a higher early embryonic survival rate, was observed (see Table 3-7). This underscores earlier suggestions that existing populations of commercial sows are increasingly resistant to negative effects of lactational catabolism on subsequent reproductive performance (Patterson *et al.,* 2011). The excellent reproductive performance in Control sows in the current trial probably explains the lack of a positive effect of marine-oil based n-3 LCPUFA supplementation on subsequent reproduction.

Our data are not consistent with earlier reports of increased litter sizes as a result of feeding n-3 LCPUFAs to gilts and sows (Webel *et al.* 2003, Spencer *et al.* 2004, Smits *et al.* 2011). One possible reason for these inconsistencies is the amount of n-3 LCPUFAs fed in the different studies, and different feedstuffs used for the basal diet. Webel *et al.* (2003) and Spencer *et al.* (2004) used a corn/soybean diet, which is higher in n-6 PUFAs than the wheat/barley based diets and adding fish oil to corn/soybean based diets may, therefore, decrease the n6:n3 ratio to a greater extent. However, Smits *et al*. (2011) also used wheat/barley in the basal diet, and the amount of n-3 LCPUFA fed per day was similar to the current trial. Another confounding factor may be the level of embryonic mortality in control sow populations in the different studies. In the study of Webel *et al.* (2004) control sows had an embryonic survival of only 59% and n-3 LCPUFA supplementation improved embryonic survival to 71%. Consistent with our results, Estienne *et al.* (2006) did not show an effect of n-3 LCPUFA supplementation on reproductive performance when embryonic survival of their control animals was already 83%. As both Webel *et al.* (2004) and Estienne *et al.* (2006) used the same marine-oil based supplement as in the current trial (Fertilium and Sow Fat Pack 10 are different

registered names for the same product), the collective data suggest that this product may improve reproductive performance when embryonic survival is a problem.

We are not aware of any previous reports on fatty acid levels in luteal tissue in pigs. Leskanich and Noble (1999) reported that ovaries and Graafian follicles contained a relatively high concentration of AA, while levels of other LCPUFA are relatively low, consistent with our findings in luteal tissue. The higher EPA and DHA and lower AA in luteal tissue in our marine-oil based n-3 LCPUFA supplemented sows may be associated with the heavier CL observed.

In the analysis of their data, Patterson *et al.* (2011) identified a subset of Restrict-fed *Risk* sows that mobilised excessive body tissues to support milk production for the nursing litter, which then had negative consequences for the quality of embryos in the subsequent litter. Similar associations were explored in the present study (Figure 3-3a and 3-3b) and suggest that even in the highly catabolic *Risk* sows, no beneficial effect of marine-oil based n-3 LCPUFA supplementation was evident either for the weight of the litter weaned or for embryonic development of the subsequent litter.

In conclusion, in the absence of an effect on litter size or birth weight, feeding gilts with a marine-oil based supplement high in n-3 LCPUFAs from day 60 of first gestation, through a 21-day lactation, tended to improve piglet body weight gain from birth until 34 days after weaning. It did not affect energy utilization by the sow during lactation and thus the catabolic state of the sows. Supplementation from weaning until day 30 of second gestation did not have an effect on overall subsequent reproductive performance, but did increase CL weight.

Table 3-1. Ingredients and calculated nutritional composition of the gestation and lactation diets (% as-fed basis) and the supplement Sow Fat Pack 10 [a]

	Gestation diet	Lactation Diet	Sow Fat Pack 10
Ingredient, %			
Wheat	.	32.17	.
Soybean Meal	.	23.10	.
Corn	.	20.00	.
Barley	50.41	13.50	.
Wheat millrun	25.00	.	.
Field peas	10.00	.	.
Corn DDGS	10.00	.	.
CL sow premix 50 [f]	.	5.00	.
Canola meal	.	5.00	.
Canola oil		1.20	.
Meat and Bone Meal	2.20	.	.
Limestone	1.40	.	.
Salt (NaCl) [b]	0.42	.	.
UF Fort #510s [e]	0.25	.	.
Biotin 100 [b]	0.15	.	.
Choline Chloride	0.07	.	.
Porzyme 9300 [c]	0.04	0.03	.
L-Lysine HCL	0.02	.	.
Vitamin E – 50000 IU/kg premix [b]	0.02	.	.
Selplex 2000 (selenium) [d]	0.01	.	.
Phyzyme 5000g (phytase) [c]	0.01	.	.
Folic acid [b]	0.01	.	.
Calculated nutrient analysis, %			
ME, (MJ/kg)	12.17	13.28	7.88
Dry matter	89.91	89.14	0.90
Crude fat	3.27	3.00	20.92
Crude protein	14.87	20.45	9.38
Calcium	0.96	1.08	0.19
Total phosphorus	0.72	0.78	0.03

Available phosphorus	0.43	0.50	0.02
True ileal digestible (TID) lysine	0.50	1.08	0.04
TID methionine : TID lysine	0.37	0.27	0.43
TID methionine + cystine : TID lysine	0.81	0.56	0.59
TID threonine : TID lysine	0.82	0.61	1.06

[a] Control and treated sows were fed with shown gestation and lactation diets, but treated sows were supplemented with 84 g/d of Sow Fat Pack 10, a marine-oil based supplement from a proprietary source (JBS United, Inc, Sheridan, IN), rich in protected n-3 LCPUFAs, as a topdressing on the shown gestation and lactation diets, from day 60 of first gestation, through a 21-day lactation period, and until d30 of second gestation

[b] Viterra, Sherwood Park, AB, Canada

[c] Danisco Animal Nutrition, Waukesha, WI, USA

[d] Alltech, Nicholasville, KY, USA

[e] Consisting of: Calcium 9.6%, phosphorus 0.2%, sodium 0.025%, magnesium 0.197%, manganese 16000 mg, zinc 52000 mg, iron 60000 mg, copper 6000 mg, selenium 120 mg, iodine 200 mg, vitamin A 4000 KIU, vitamin D 600 KIU, vitamin E 24 KIU, vitamin K 900 mg, thiamin 600 mg, riboflavin 3200 mg, niacin 18000 mg, pyridoxine 800 mg, pantothenate 10000 mg, choline 259 mg, vitamin B12 12 mg, folic acid 300 mg, biotin 60 mg, ethoxyquin 500 mg, selenium 60 mg; Viterra, Sherwood Park, AB, Canada

[f] Consisting of: dry matter 96%, crude fat 1.78%, crude fibre 2.69%, crude protein 8,54%, lysine 3.20%, methionine 0.075%, methionine and cystine 0.186%, threonine 0.15%, tryptophan 0.068%, arginine 0.282%, app ME enzyme 785.9 Kcal, calcium 19.5%, phosphorus 7.4%, sodium 4.1%, salt 10.35%, copper 304.9 mg, selenium 6.0 mg, vitamin A 2000 KIU, vitamin D 30 KIU, vitamin E 1.6 KIU, thiamine 35 mg, riboflavin 160.5 mg, niacin 903.4 mg, pyridoxine 40 mg, pantothenate 504.4 mg, choline 8276.7 mg, vitamin B12 0.6 mg, folic acid 40.4 mg, biotin 12.2 mg, linoleic acid 0.588%; Viterra, Sherwood Park, AB, Canada

Table 3-2. Fatty acid composition (% of total fatty acids) of the LCPUFA supplement (Sow Fat Pack 10) at the end of each of the 2 batches used

Fatty acid	Batch 1	Batch 2
Total fat	19.80	19.28
C14:0	8.41	7.92
C14:1	0.14	0.08
C15:0	0.76	0.70
C16:0	18.91	18.45
C16:1	11.15	10.57
C17:0	0.53	0.62
C17:1	1.49	1.40
C18:0	3.27	3.07
C18:1 t9	1.43	1.49
C18:1 n9	7.89	7.33
C18:1 n7	3.06	3.43
C18:2 n6	5.44	5.90
C18:3 n3	1.75	2.01
C18:4 n3	2.49	2.59
C20:0	0.19	0.19
C20:1 n9	0.99	0.94
C20:3 n3	0.00	0.17
C20:4 n6	0.82	0.95
C20:4 n3	1.24	1.30
C20:5 n3	12.09	11.48
C22:0	0.00	0.11
C22:1 n9	0.00	0.18
C22:5 n3	2.13	2.09
C22:6 n3	9.41	10.05
C24:0	0.00	0.00
C24:1 n9	0.28	0.44
Undefined	6.11	6.57

Data are the means

Table 3-3. Matrix used to determine the feed allowance in kg/day in gestation

Breeding Wt (kg)	Backfat Measurement (P2) in mm at breeding								
	12	13	14	15	16	17	18	19	20
115-120	2.4	2.3	2.2	2.2	2.1	2.1	2.0	2.0	2.0
121-130	2.4	2.3	2.2	2.2	2.1	2.1	2.0	2.0	2.0
131-140	2.5	2.4	2.3	2.2	2.1	2.1	2.0	2.0	2.0
141-150	2.6	2.5	2.3	2.3	2.2	2.2	2.1	2.1	2.0
151-160	2.7	2.6	2.5	2.4	2.4	2.3	2.2	2.2	2.1
161-170	2.8	2.7	2.6	2.5	2.4	2.4	2.3	2.2	2.2
171-180	2.9	2.8	2.7	2.6	2.5	2.4	2.4	2.3	2.3
181-190	2.9	2.9	2.8	2.7	2.6	2.5	2.4	2.4	2.3
191-200	3.0	3.0	2.9	2.8	2.7	2.6	2.5	2.4	2.4

Table 3-4. Ingredients and calculated nutritional composition of the nursery diets (% as-fed basis), fed to all piglets on trial [a]

	Phase 1	Phase 2	Phase 3	Pre-grower
Ingredient, %				
Oat highpro by-product	19.94	10.00	5.90	.
Wheat	18.40	46.38	59.97	35.84
Wheat millrun	.	.	.	15.00
Wheat DDGS (Husky) [h]	.	3.00	5.00	.
High lactose whey	18.07	12.50	.	.
Soybean meal	15.00	16.80	12.30	12.40
Corn	.	.	9.40	.
Corn DDGS	.	.	.	15.00
Field peas	.	.	.	7.50
Canola meal	.	.	.	5.50
Fish meal, BC herring	8.50	3.75	.	.
Animal plasma 920	6.00	.	.	.
Fat, blend tallow	6.00	1.50	.	.
Fat, downstream	.	2.50	2.50	1.80
Lactose, edible	5.00	.	.	.
Limestone	0.80	1.50	1.50	1.92
Zinc oxide 72%	0.40	.	.	.
Bioplex Zinc 15%	.	0.17	0.17	.
L-Lysine HCL	0.35	0.52	0.83	0.44
Salt (NaCl) [b]	.	0.05	0.47	0.39
MHA (alimet)	0.26	0.20	0.27	0.12
UF Fort #510S-03 [b]	0.25	0.25	0.25	0.25
Tetracid 500 [f]	0.20	0.10	0.10	.
Threonine-L	0.17	0.22	0.33	0.15
Tryptophan-L	.	0.01	0.04	.
Vitamin E – 50000 IU/kg premix	0.12	0.10	0.05	.
Bio-mos [d]	0.10	0.20	0.20	.
Lincomycin + spectinomycin	0.10	.	.	.
Hyperegg K88 [g]	0.10	.	.	.
Water for enzyme application	0.08	0.08	0.08	0.08

Choline, liquid 70%	0.07	0.07	0.07	0.04
Flavour, maxi-gro [e]	0.05	0.05	0.05	.
Bioplus 2B [c]	0.04	0.04	0.04	.
Liquid Xylanase [e]	0.02	0.02	0.02	0.02
Dical 21% (412) macro	.	.	0.43	.
Copper Sulfate	.	.	0.04	0.04
Extrapro	.	.	.	3.50
Ethoxyquin 66%	.	.	.	0.02
Calculated nutrient analysis, %				
ME, (MJ/kg)	14.97	14.25	13.97	13.68
Dry matter	91.49	89.69	88.79	89.48
Crude fat	8.41	5.79	4.28	5.44
Crude protein	24.10	20.25	18.12	21.50
Calcium	1.01	1.01	0.80	0.94
Total phosphorus	0.75	0.60	0.57	0.66
Available phosphorus	0.59	0.40	0.35	0.36
True ileal digestible (TID) lysine	1.64	1.24	1.20	1.12
TID methionine : TID lysine	0.35	0.37	0.37	0.34
TID methionine + cystine : TID lysine	0.58	0.60	0.60	0.62
TID threonine : TID lysine	0.63	0.65	0.65	0.67

[a] Phase 1 diet was fed at 0.3 kg/pig. Phase 2 diet was fed at 5 kg/pig. Phase 3 diet was fed ad libitum from completion of Phase 2 up until 8 weeks of age. Finally, the pre-grower diet was fed ad libitum for the final week of the nursery stage

[b] Consisting of: Calcium 9.6%, phosphorus 0.2%, sodium 0.025%, magnesium 0.197%, manganese 16000 mg, zinc 52000 mg, iron 60000 mg, copper 6000 mg, selenium 120 mg, iodine 200 mg, vitamin A 4000 KIU, vitamin D 600 KIU, vitamin E 24 KIU, vitamin K 900 mg, thiamin 600 mg, riboflavin 3200 mg, niacin 18000 mg, pyridoxine 800 mg, pantothenate 10000 mg, choline 259 mg, vitamin B12 12 mg, folic acid 300 mg, biotin 60 mg, ethoxyquin 500 mg, selenium 60 mg; Viterra, Sherwood Park, AB, Canada

[c] Chr Hansen, Milwaukee, WI, USA

[d] Derived from a specific strain of yeast; Alltech, Nicholasville, KY, USA

[e] Canadian Biosystems, Calgary, AB, Canada

[f] Combination of protected acids and nature identical compounds; Jefo, St. Hyachinthe, QC, Canada

[g] Spray-dried whole egg powder containing polyclonal antibodies to E. Coli K88; J.H. Hare and Assoc., Winnipeg, MB, Canada

[h] Husky Energy, Lloydminster, AB, Canada

Table 3-5. Effect of n-3 LCPUFA supplementation in gilts on body weight and backfat measurements [a, b]

Item	CON	mLCPUFA	S.E.D.	P-value
Sow weights (kg) [c]				
D60 gestation	166 (n=52)	166 (n=54)	0.6	0.55
Farrowing	179 (n=49)	181 (n=47)	1.3	0.08
Wn-7 [d]	177 (n=44)	179 (n=40)	1.4	0.37
Weaning	161 (n=47)	161 (n=44)	1.7	0.96
Breeding	169 (n=33)	168 (n=33)	1.7	0.53
D30 gestation	181 (n=43)	183 (n=42)	1.5	0.21
Sow Backfat (mm) [e]				
D60 gestation	15.8 (n=52)	15.5 (n=55)	0.6	
Farrowing	16.3 (n=47)	16.4 (n=47)	0.5	
Wn-7	14.2 (n=49)	14.7 (n=44)	0.5	
Weaning	12.4 (n=48)	12.7 (n=44)	0.5	
Breeding	13.8 (n=32)	13.6 (n=34)	0.6	
D30 gestation	14.9 (n=44)	14.3 (n=43)	0.5	

[a] Gilts were fed standard gestation and lactation diets with (LCPUFA) or without (CON) 84 g/day of a LCPUFA rich supplement from day 60 (D60) of gestation onwards. Data are the least square means. S.E.D.: Standard error of the difference

[b] Due to loss of some barn record sheets of sows on experiment between farrowing and breeding, data are based on the number of animals shown

[c] P=0.05 for the interaction between treatment and time

[d] Wn-7: the 7-days period before weaning when all sows were feed restricted

[e] P=0.89 for Treatment, P<0.001 for Time and P=0.27 for treatment by time interaction

Table 3-6. Lack of an effect of marine-oil based n-3 LCPUFA supplementation in gilts on litter characteristics at birth

Item	CON	mLCPUFA	RSD	P-value
n	52	52		NS
Born alive	13.1	13.4	2.2	NS
Stillborn	0.4	0.5	0.8	NS
Total born	13.5	13.8	2.1	NS
Mummies	0.3	0.4	0.6	NS
LitBW (kg)	1.3	1.3	0.2	NS
litvarBW (g)	216	214	61	NS

Gilts were fed standard gestation and lactation diets with (mLCPUFA) or without (CON) 84 g/day of a marine-oil based LCPUFA supplement from day 60 of gestation onwards.
Data are the least square means. RSD: residual standard deviation
LitBW: litter average birth weight, litvarBW: within-litter variation in birth weight

Table 3-7. Effect of marine-oil based n-3 LCPUFA supplementation in gilts on subsequent reproductive performance after weaning

Item	CON	mLCPUFA	RSD	P-value
WEI (days)	5.3 (n=47)	5.3 (n=46)	1.2	NS
Breeding rate (% of sows weaned)	97.9 (n=48)	95.8 (n=48)	. [a]	NS
Pregnancy rate (% of sows bred)	95.7 (n=47)	93.5 (n=46)	. [a]	NS
Day of gestation at euthanasia	30.2 (n=45)	30.3 (n=43)	0.7	NS
Ovulation rate	20.6 (n=45)	20.3 (n=43)	2.6	NS
No. live embryos	16.2 (n=45)	15.6 (n=43)	3.2	NS
Embryonic survival (%)	78.9 (n=45)	76.6 (n=43)	13.0	NS
Embryonic weight (g)	1.69 (n=44)	1.68 (n=42)	0.30	NS
Embryo crown-rump length (mm)	26 (n=44)	25 (n=42)	1	NS
Allantochorionic fluid volume (ml)	223 (n=43)	227 (n=42)	38	NS
Average CL weight (mg)	341 (n=43)	376 (n=42)	46	<0.001

Gilts were fed standard gestation and lactation diets with (mLCPUFA) or without (CON) 84 g/day of a marine-oil based LCPUFA supplement from day 60 of gestation onwards. Data are the least square means. RSD: residual standard deviation

[a] RSD not available for categorical data (proc catmod in SAS)

Table 3-8. Effect of marine-oil based n-3 LCPUFA supplementation in gilts on fatty acid composition (as % of total fatty acids) of sow serum (d110 of first gestation and d30 of second gestation), embryos and CL (d30 of second gestation)

	Serum at d110 of gestation				Serum at day 30 of gestation				Embryos at d30 of gestation				CL at d30 of gestation			
Fatty acid	CON	mLC PUFA	RSD	P-value	CON	mLC PUFA	RSD	P-value	CON	mLC PUFA	RSD	P-value	CON	mLC PUFA	RSD	P-value
n	10	9			9	10			10	10			10	10		
unknown	2.7	2.7	1.0	0.90	1.4	0.9	0.3	0.01	20.9	20.9	1.2	0.99	2.9	2.7	0.2	0.14
SFA [a]	28.2	26.9	1.4	0.07	32.3	30.1	2.0	<0.05	41.7	42.1	0.9	0.41	40.0	39.6	1.3	0.54
MUFA [b]	24.7	23.2	2.5	0.23	20.6	18.0	1.6	<0.01	29.9	29.3	1.0	0.16	9.7	9.6	0.6	0.84
PUFA [c]	44.4	47.1	2.7	0.06	45.6	51.0	3.5	<0.01	7.4	7.7	1.5	0.66	47.5	48.1	1.8	0.47
C18:2 n-6	33.7	33.5	2.3	0.86	33.0	36.8	2.1	<0.01	0.7	0.7	0.2	0.31	16.3	16.9	0.8	0.15
C18:3 n-6	0.3	0.2	0.1	0.05	0.3	0.2	0.1	<0.01	ND[d]	ND[d]	.	.	0.09	0.04	0.07	0.15
C20:3 n-6	0.3	0.4	0.1	0.10	0.3	0.4	0.1	0.14	0.2	0.1	0.1	0.49	0.9	1.0	0.1	<0.01
C20:4 n-6	5.5	3.6	0.9	<0.01	8.3	5.6	1.5	<0.01	3.9	3.3	0.7	0.08	21.4	20.0	0.9	0.01
C18:3 n-3	2.2	2.4	0.3	0.26	1.0	1.6	0.3	<0.01	0.3	0.2	0.1	0.22	0.3	0.4	0.1	0.31
C20:5 n-3	0.3	3.0	0.4	<0.001	0.3	2.5	0.5	<0.001	0.2	0.4	0.4	0.26	ND[d]	0.5	0.1	<0.001
C22:5 n-3	1.2	1.5	0.3	<0.05	1.4	1.7	0.5	0.20	ND[d]	0.02	0.04	0.34	1.3	2.5	0.3	<0.001
C22:6 n-3	0.3	2.1	0.3	<0.001	0.3	1.9	0.4	<0.001	1.8	2.7	0.7	<0.01	0.1	1.0	0.2	<0.001
Total n-6	39.8	37.7	2.7	0.13	42.0	43.0	2.7	0.45	4.8	4.1	0.9	0.10	38.7	38.0	1.1	0.22
Total n-3	3.9	9.0	0.7	<0.001	3.0	7.7	1.0	<0.001	2.2	3.3	0.9	0.01	1.7	4.4	0.5	<0.001
n-6 : n-3	10.2	4.2	1.0	<0.001	15.3	5.7	3.6	<0.001	2.3	1.3	0.4	<0.001	22.8	8.9	2.6	<0.001

Gilts were fed standard gestation and lactation diets with (mLCPUFA) or without (CON) 84 g/day of a marine-oil based LCPUFA supplement from day 60 of first gestation onwards. Data are the least square means. RSD: residual standard deviation

[a] SFA: Saturated fatty acids, [b] MUFA: Mono-unsaturated fatty acids, [c] PUFA: polyunsaturated fatty acids, [d] ND: non detectable

Table 3-9. Effect of marine-oil based n-3 LCPUFA supplementation in gilts on fatty acid composition (in mg/l serum or mg/kg tissue) of sow serum (d110 of first gestation and d30 of second gestation), embryos and CL (d30 of second gestation)

	Serum at d110 of gestation				Serum at d30 of gestation				Embryos at d30 of gestation				CL at d30 of gestation			
Fatty acid	CON	mLC PUFA	RSD	P-value	CON	mLC PUFA	RSD	P-value	CON	mLC PUFA	RSD	P-value	CON	mLCPUFA	RSD	P-value
n	10	9			9	10			10	10			10	10		
unknown	36.2	41.9	29.6	0.69	12.8	9.5	3.7	0.07	597.9	628.6	98.2	0.49	181.0	145.8	61.3	0.22
SFA [a]	325.2	327.0	75.5	0.96	293.6	320.8	86.1	0.51	1195.7	1263.0	186.3	0.43	7921.7	7498.6	1336.4	0.49
MUFA [b]	281.5	281.5	95.9	0.99	185.5	193.4	58.2	0.78	855.0	880.8	124.5	0.65	1913.3	1810.8	327.3	0.49
PUFA [c]	512.8	559.1	84.1	0.27	412.5	538.9	132.3	0.05	214.9	234.1	62.1	0.50	9382.6	9124.9	1595.1	0.72
C18:2 n-6	389.6	395.3	64.9	0.86	299.4	390.2	102.0	0.07	21.4	20.3	5.8	0.70	3243.0	3200.2	617.2	0.88
C18:3 n-6	3.5	2.8	0.9	0.13	3.0	2.4	0.6	0.08	ND[d]	ND[d]	.	.	17.5	7.9	14.2	0.16
C20:3 n-6	3.4	4.2	0.7	<0.05	2.9	4.0	0.9	<0.05	4.9	3.7	4.4	0.57	171.0	198.3	44.3	0.20
C20:4 n-6	64.1	42.7	10.5	<0.01	74.0	57.9	19.1	0.08	111.5	99.1	26.9	0.32	4223.1	3802.6	720.4	0.21
C18:3 n-3	25.1	29.3	9.2	0.35	9.2	17.7	6.5	0.01	8.0	6.5	3.0	0.30	66.0	69.0	13.8	0.64
C20:5 n-3	3.1	36.8	10.1	<0.001	2.4	26.2	6.2	<0.001	5.8	12.8	11.7	0.21	ND[d]	88.1	19.5	<0.001
C22:5 n-3	13.5	18.1	3.4	<0.05	12.4	17.3	5.0	<0.05	ND[d]	0.5	1.0	0.34	258.8	478.1	85.9	<0.001
C22:6 n-3	2.8	26.1	6.6	<0.001	2.7	19.7	5.5	<0.001	51.1	82.1	26.1	<0.05	15.5	189.1	28.5	<0.001
Total n-6	460.6	444.5	68.8	0.63	379.2	454.5	115.2	0.17	137.7	123.2	34.0	0.35	7654.6	7209.1	1345.6	0.47
Total n-3	44.5	110.2	24.8	<0.001	26.7	80.9	18.5	<0.001	64.8	101.8	32.7	<0.05	340.3	824.3	133.8	<0.001
n-6 : n-3	10.3	4.2	1.0	<0.001	15.3	5.7	3.6	<0.001	2.3	1.3	0.4	<0.001	22.8	8.9	2.6	<0.001

Gilts were fed standard gestation and lactation diets with (mLCPUFA) or without (CON) 84 g/d of a marine-oil based n-3 LCPUFA supplement from day 60 of gestation onwards. Data are the least square means. RSD: residual standard deviation

[a] SFA: Saturated fatty acids, [b] MUFA: Mono-unsaturated fatty acids, [c] PUFA: polyunsaturated fatty acids, [d] ND: non-detectable

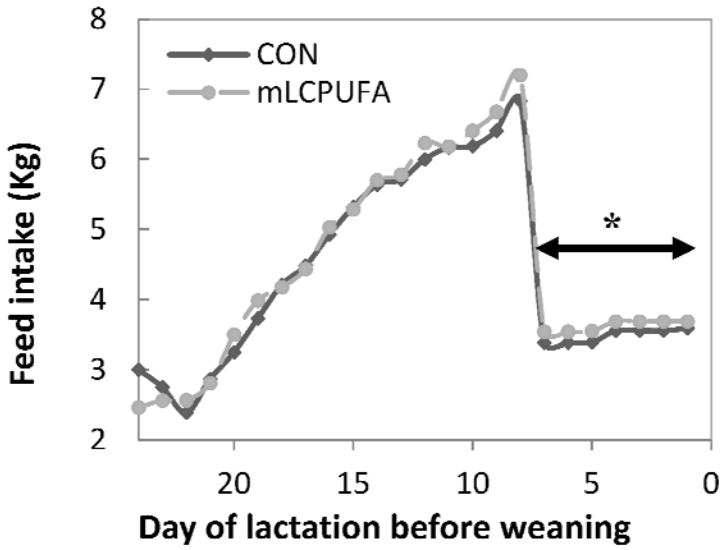
Table 3-10. Effect of marine-oil based n-3 LCPUFA supplementation in gilts on energy input and output during lactation

Item (MJ/day)	CON (n=40)	mLCPUFA (n=35)	RSD	P-value
Farrowing to Wn-7[a]				
Input energy from the sow				
ME intake from feed consumed	67.1	67.9	8.1	0.66
Energy from tissue mobilisation	13.9	10.2	9.0	0.10
Energy required for sow maintenance	-21.2	-21.7	0.5	<0.001
Net input (energy) from the sow	59.8	56.3	11.3	0.20
Energy output to the litter				
Energy for litter maintenance	10.5	10.5	1.1	0.98
Energy for litter growth	24.0	24.5	3.9	0.65
Total output to the litter	34.5	34.9	4.9	0.72
Lactational efficiency (%) [b]	59.7	65.1	15.8	0.15
Wn-7 to Wean[a]				
Input energy from the sow				
ME intake from feed consumed	46.2	47.4	2.8	0.09
Energy from tissue mobilization	36.5	39.5	16.4	0.45
Energy required for sow maintenance	-20.4	-20.8	0.5	<0.01
Net input (energy) from the sow	62.3	66.1	16.9	0.36
Energy output to the litter				
Energy for litter maintenance	15.5	15.5	1.5	0.90
Energy for litter growth	25.5	25.9	3.3	0.61
Total output to the litter	41.2	41.4	3.5	0.85
Lactational efficiency (%) [b]	74.9	69.9	26.0	0.44

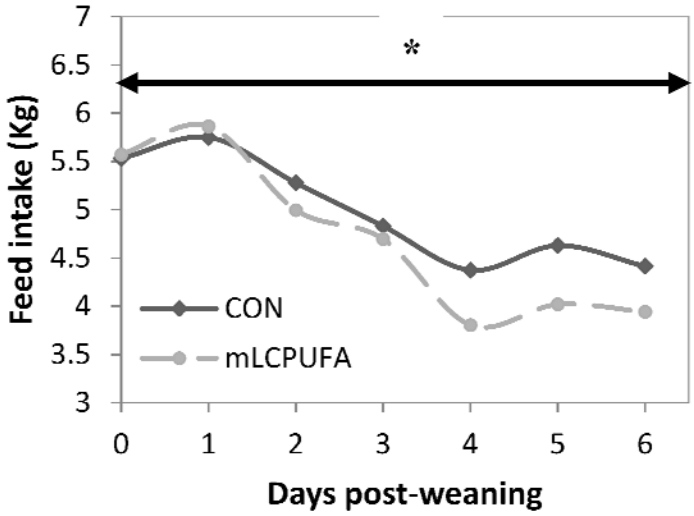
Gilts were fed standard gestation and lactation diets with (mLCPUFA) or without (CON) 84 g/d of a marine-oil based n-3 LCPUFA supplement from day 60 of gestation onwards. Data are the least square means. RSD: residual standard deviation

[a] Wn-7: the last seven days before weaning, at which time feed restriction was implemented to all sows

[b] Defined as energy efficiency of sows during lactation and calculated as follows: output x 100 / input (Bergsma *et al.*, 2009)



a.



b.

Figure 3-1. Feed intake during lactation (Figure 3-1a: n=47 for CON and 43 for mLCPUFA sows) and feed intake during rebreeding (Figure 3-1b: n=42 for CON and 42 for mLCPUFA sows) for sows fed standard gestation and lactation diets with (mLCPUFA) or without (CON) 84 g/day of a marine-oil based LCPUFA supplement from day 60 of first gestation onwards. All sows were fed according to a step-up regimen from farrowing until 7 days before weaning and were then feed restricted for the last 7 days until weaning.

* Significant difference between CON and LCPUFA sows during the last week of lactation and during the rebreeding period ($P<0.05$)

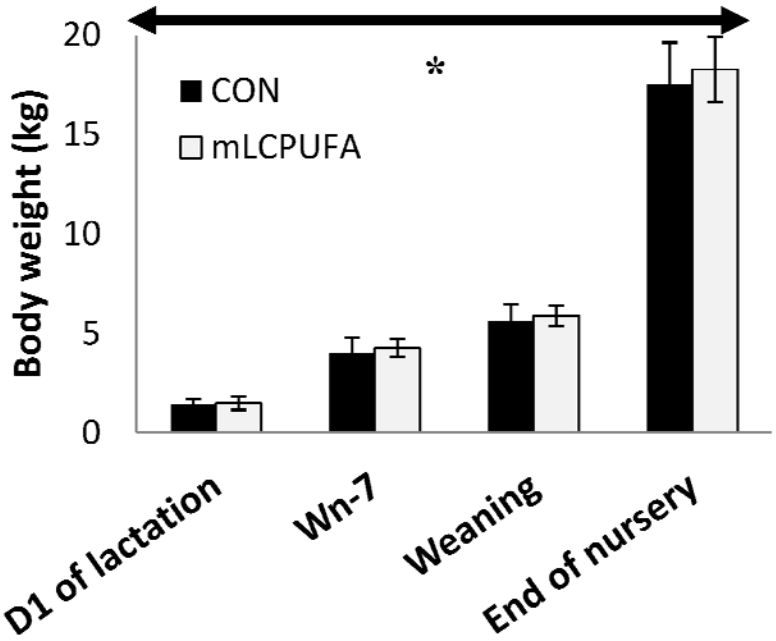
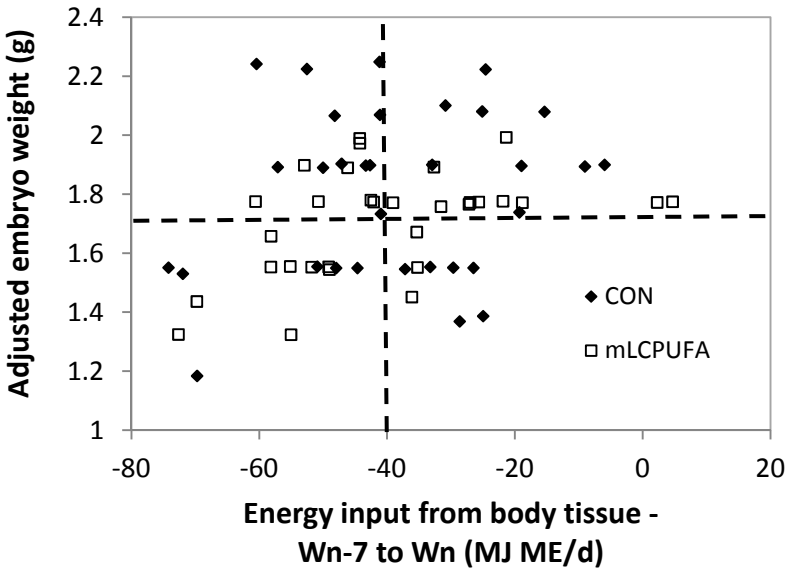
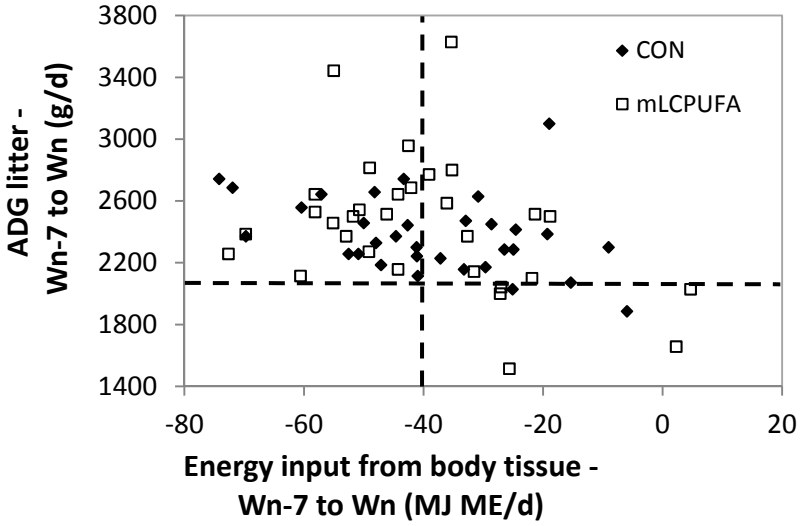


Figure 3-2. Average piglet weight per litter (kg) at different time points from birth to the end of the nursery period (Wn-7: 7 days before weaning) for sows fed standard gestation and lactation diets with (mLCPUFA) or without (CON) 84 g/day of a marine-oil based LCPUFA supplement from day 60 of first gestation until weaning. Bars represent the means.

* P-value for Treatment: 0.06 and for Time: <0.001. No significant interaction.



a.



b.

Figure 3-3. Overall relationship between sow energy input from body tissue mobilisation during the last 7 days of lactation (Wn-7: 7 days before weaning, Wn: weaning) and a) adjusted (by day of gestation) embryo weight, and b) average daily gain (ADG) of the litter, for sows fed standard gestation and lactation diets with (mLCPUFA) or without (CON) 84 g/day of a marine-oil based LCPUFA supplement from day 60 of first gestation onwards. Thresholds for "Risk" sows is set at -40 MJ ME/day (see Patterson *et al.*, 2011): The mean population adjusted embryo weight is shown at 1.76 g (Figure 3-3a) and the threshold for litter daily weight gain is set at ~2100 g/day (Figure 3-3b).

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Chapter 4: Associations among litter birth weight phenotype, post-natal lean growth performance and testicular development in the pig [2]

4.1 Introduction

A disproportional increase in ovulation rates in higher parity sows, linked to good or reasonable (>60%) embryonic survival (Vonnahme *et al*., 2002), are positively associated with increased numbers of embryos in the uterus at day 30, which negatively influences placental weight at day 30 and 50 of gestation (Patterson *et al*., 2008). Uterine capacity then becomes a critical constraint on both litter size and quality (Foxcroft *et al.*, 2009), reducing fetal weights at day 50 (Patterson *et al.,* 2008), and by inference birth weight, in sows with high ovulation rates. In contrast, higher parity sows with lower ovulation rates or poorer embryonic survival will have a lower risk of developmental problems and the litters of these sows will have higher birth weights. Moreover, Town *et al*. (2004) found that pigs from relative crowded uteri (15 fetuses at day 90 of gestation) showed lower placental weights and higher brain:liver weight ratios than pigs from less crowded uteri (9 fetuses at day 90 of gestation). Subsequently, (Smit, 2007) reported that low average birth weight litters of 10 to 15 pigs born total had more pigs born dead and less pigs weaned, suggesting reduced viability in these litters.

The underlying concept for the present study was, therefore, that after accounting for the predicted effect of increased numbers of pigs born on birth weight, the large residual variance in litter average birth weight in litters of 9 to 16 pigs born reflects the negative effects of relative intrauterine crowding driven by a high ovulation rate phenotype. Low average birth weight litters should, therefore, show the development benchmarks associated with intra-uterine growth restriction (**IUGR**). Based on extensive comparisons of postnatal performance in low and high birth weight pigs within a litter (Quiniou *et al*., 2002; Gondret *et al*., 2006; Fix *et al*., 2010), it is also predicted that IUC and a low litter birth weight phenotype will negatively affect lean growth performance of litters after weaning.

Effects of a low litter birth weight phenotype on male fertility have not been studied to-date. It is known that Sertoli cell proliferation in pigs begins during the prenatal period

[2] A version of this chapter has been submitted for publication in Animal

(McCoard *et al.*, 2002) and continues after birth (Swanlund *et al.*, 1995; França *et al.*, 2000). The total number of Sertoli cells achieved will determine testicle size in adulthood, as well as the sperm production capacity (Cooke *et al.*, 1992; Hess *et al.*, 1993). Therefore, programming effects of IUGR on the testes may negatively affect male lifetime fertility.

Given this background, the specific objectives of this trial were to investigate effects of high versus low litter average birth weight phenotype on post-natal lean growth performance, carcass quality, and neonatal testicular morphology in male offspring.

4.2 Material and Methods

4.2.1 Animals and Treatments

This study was performed according to Canadian Council on Animal Care and JBS United Inc. ethical guidelines. Multiparous Large White x Landrace terminal line sows (Camborough; PIC, Nashville, TN, USA) were managed according to approved protocols at the JBS United Inc. research facilities (Sheridan, IN, USA). A total of 223 sows, with information on litter average birth weight of the preceding litter, and that farrowed within 5 successive weekly breeding groups in the summer of 2009 at the JBS Bache research facility, were used. After weaning, sows were rebred within 8 days and 168 sows farrowed again in the winter of 2009. Another 25 sows that farrowed in the winter of 2009 in the same breeding groups but that did not have information on litter average birth weight of preceding litters available, were also used for this trial. Sows ranged between parity 2 and 8 (mean = 4.6 ± 1.1). All sows were fed standard corn/soybean meal based gestation and lactation diets (Table 4-1).

Both in the summer and winter, individual birth weight of all pigs born was measured within 24 hours after birth. Litter average birth weight was calculated as total birth weight of all pigs in a litter divided by the total number of pigs born in that litter. Because extremes of high litter size will inevitably reduce both the mean and variation in litter birth weight, only litters between 9 and 16 total born were used in the analysis and for the nursery and grow-finish trials (Table 4-2). This also ensured that the number of pigs after cross-fostering were even between birth weight categories. Each litter between 9 and 16 total pigs born was then classified as low (**LBW**), medium (**MBW**) or high (**HBW**) birth weight as shown in Table 4-2. HBW and LBW was defined as litter average birth weight being more than one standard deviation above and below the population litter average

birth weight for each litter size, based on data from the preceding 2 farrowings of the sows on trial. Medium birth weight was defined as litters being less than one standard deviation above or below this population mean for each litter size.

4.2.2 Measurements in summer 2009

-At birth:

Within 24 hours after birth and before cross-fostering, Sow ID, parity, date of birth, total number of piglets born, number of piglets born alive, number of stillborns, number of mummies, individual birth weight (of all pigs born) and sex (of all pigs born) were recorded.

Stillborn piglets or piglets that died shortly after birth from any litter were dissected within 24 hours after birth. Stillbirth was confirmed by removing the lungs and conducting a "lung floatation" test to determine if the piglets were born dead and never breathed (lungs not floating) or if they were born live but died soon after birth (lungs floated). Stillborns that were smaller than 2 standard deviations below their litter average birth weight (i.e. runts) were not dissected. The measurements taken at necropsy were brain weight, liver weight, small intestine weight and wet weight of the *Semitendinosus* muscle of the right leg.

-Testicular data [3]

Forty male pigs, born to different 4^{th}- 6^{th} parity sows and in litters of 10 to 15 pigs born in total, and identified as falling into high (**HW**: range 1.8 to 2.2 kg and litter average birth weight of 1.87 ± 0.09 kg) and low (**LW**: range 0.8 to 1.2 kg and litter average birth weight of 1.13 ± 0.05 kg) birth weight categories, were castrated at 5.5 ± 1.3 days of age. Both body (**BdW**) and testicular (**TW**) weights were measured, and the gonadosomatic index (**GSI** = TW/BdW x 100) was calculated. Fresh transverse sections of the testes were fixed and stored in 5% glutaraldehyde (EMS biological grade) in 0.05 M sodium phosphate buffer (pH 7.2-7.4). Testes tissue samples were subsequently processed by washing in three changes of buffer and embedded in glycol methacrylate plastic resin (Leica, Historesin). Histological sections (5 µm) were cut from these resin blocks and stained with toluidine blue-borate for histomorphometric analysis (Chiarini-Garcia *et al.*,

[3] The testicular development data was a collaboration with the Universidade Federal de Minas Gerais, led by Professor F.R.C.L Almeida

2011). The absolute numbers of germ cells, Sertoli cells and Leydig cells in the entire testis were estimated as described by Sinha Hikim *et al.* (1988) and Drumond *et al.* (2011). Briefly, 10 randomly selected sections per animal in each group were examined under a binocular BX-51 microscope equipped with a bright field condenser with a 40X objective, and the nuclei of each cell type were counted. The absolute numbers of germ cells, Sertoli and Leydig cells were then divided by testicular weight to provide results expressed as an index relative to testicular weight (Cellular-gonadal index).

4.2.3 Measurements and management before weaning in winter 2009

At birth the same measurements were taken as in summer 2009. A maximum of 2 male and 2 female stillborn pigs per litter were necropsied, as described above. In addition, the number and total wet weight per litter of all placentae recovered was recorded, from which litter average placental wet weight was calculated: However, placental data were only included in subsequent analyses when more than 50% of the placentae in a litter were recovered, which occurred in 89 litters.

All piglets in LBW and HBW litters with between 9 and 16 total pigs born were ear-tagged at birth, and piglets from MBW litters were ear-tagged the day before weaning. Cross-fostering of tagged litters only occurred within birth weight classification, but non-tagged piglets born to sows not included in the study could be cross-fostered into a tagged litter if needed. When a tagged pig died, the date of death and weight were recorded. All pigs were weighed on the day before weaning.

4.2.4 Management after weaning (winter 2009 only)

From the first two breeding groups farrowed, HBW, MBW and LBW litters were randomly selected for study in the nursery and grow-finish periods at the JBS Burton Russell research facility, with selected litters providing 13 male and 13 female progeny from the same birth weight category to fill a single nursery pen. In the last three breeding groups farrowed, all litters were selected to be followed in the nursery and grow-finish period in order to fill as many pens as possible for each birth weight category in the nursery facility, again with 13 males and 13 females per pen randomly selected from the available litters. Pigs weighing less than 2.7 kg at weaning were excluded from selection (6 LBW pigs, 1 MBW pig and 3 HBW pigs). In total, 9 HBW, 17 MBW and 10 LBW pens were established in the nursery using an incomplete block design, with blocks based

on pens. Pens were divided over two nursery barns, but pens in a block were located in the same barn. In the nursery, pigs had a space allowance of 0.38 m^2. At 6 weeks after weaning pigs were moved to two grow-finish barns, keeping the same pigs in a pen as in the nursery. In the grow-finish phase, pigs had a space allowance of 0.62 m^2.

Pigs of all birth weight categories were fed using a commercial 4-phase nursery program (JBS United Inc., Sheridan, IN; Table 4-3) for the first 6 weeks after weaning. The nursery phase 1 diet consisted of a pelleted diet fed for the first week, followed by meal diets fed for one, one and 3 weeks, respectively, for nursery phases 2 through 4. The common grow-finish diets (JBS United Inc., Sheridan, IN; Table 4-3) were corn and soybean meal based. Each grow-finish phase diet was fed for 21 days until pigs were marketed. All diets were formulated to be above all NRC nutritional requirements (NRC, 1998), were supplemented with 3-4% choice white grease, and the lysine:ME ratio was at 105% of the experimentally determined requirements for the genotype used in this trial. The additional energy and amino acids were provided to allow potential differences in lean protein deposition among birth weight categories to be expressed. Pigs were shipped by pen to a commercial slaughterhouse (Tyson, Logansport, IN, USA) at a targeted live market weight of 117 kg.

4.2.5 Measurements after weaning (winter 2009 only)

Pigs were weighed on a pen basis (not individually) within 24 hours after weaning, then weekly during the 6-week nursery period and once every 4 weeks during the grow-finish period. Pigs were weighed individually the day before slaughter. Average daily feed intake, mortality and morbidity, and scour scores were measured on a pen basis throughout the nursery and grow-finish periods. Carcass data were also recorded on a pen basis.

4.2.6 Statistical analysis

All variables were tested for normality prior to analyses, using the univariate procedure in SAS. Also the homogeneity of variance of the residuals was tested for each variable, using the Bartlett and Levine's tests in SAS. When variables were not normally distributed, they were only transformed if the variance of residuals was not homogeneous and if it improved the fit of the model. None of the variables needed transformation on this basis.

For the testicular data, data were analyzed as a randomized design, and the statistical model included birth weight class (HW and LW) as fixed effect and piglet as a random effect. Treatment effects on castration weight, testes weight, GSI, absolute cell numbers, cellular-gonadal indexes and cell number per gram of testes were analyzed using the general linear model (GLM) procedure of SAS. Least square means were compared using the Student T test. Important associations among body weight, testicular weight and Sertoli cell number were examined across treatment groups using correlation analysis (INSIGHT procedure of SAS).

For all parameters at birth, litter was used as the experimental unit, whereas pen was used as the experimental unit for all parameters tested after weaning. Data were analyzed using the MIXED procedure of SAS. Data of stillborn piglets was averaged by litter, and associations between piglet birth weight and tissue weights were analyzed across treatment groups using regression analysis.

After weaning, a randomized incomplete block design was used, with blocks based on pens. In the case of unequal numbers of pens for each litter birth weight category, an incomplete block was formed with one or two birth weight categories present. The model included litter average birth weight category (LBW, MBW, HBW) as a fixed effect and block as a random effect.

Repeated measures analysis was used for pen weight, pen feed intake and feed utilization efficiency after weaning. An appropriate covariance structure was selected by comparing the goodness-of-fit measures of different structures. The Kenwardroger approximation was used for the denominator degrees of freedom. Categorical data like scour scores and mortality were analyzed separately using the generalized logit function (proc CATMOD in SAS).

Repeatability of litter average birth weight within sows was analyzed using correlation analysis between litter average birth weight of consecutive litters within sows.

Data in the text are given as least square means ± S.E.M., unless otherwise stated. In the tables, data are reported as least square means and residual standard deviation and data in the figures as means. Probability values < 0.05 were considered significant and values < 0.10 were used to describe trends.

4.3 Results

4.3.1 Testicular data

Testes weight and body weight at castration were different (P<0.01) for HW and LW males, respectively (Table 4-4), while testis weight relative to body weight (GSI) was similar. When brain weight at birth was calculated with the formula given in Figure 4-4, the brain:testes weight ratio was shown to be higher (P<0.001) for LW compared to HW males (Table 4-4). The diameter of the seminiferous tubules was not affected by birth weight (56.8 ± 0.4 µm vs. 56.7 ± 0.5 µm for HW and LW piglets, respectively, RSD: 5.49 µm, P > 0.05). The histomorphometrical analysis established that LW males had lower absolute numbers of germ cells, Sertoli cells and Leydig cells than HW males (P < 0.01; Table 4-4 and Figure 4-1), but the numbers of cells per gram of testes were similar between HW and LW animals. Figure 4-1 also shows that Leydig cells are partially replaced by adipocytes in the interstitial tissue of testes from the LW males. Overall, testicular weight was positively correlated to body weight, and Sertoli cell number was positively correlated with testicular weight and body weight (Table 4-4).

4.3.2 Birth data

Of the 192 sows farrowing, 18 sows gave birth to a litter with less than 9 pigs in total, 148 sows had a litter between 9 and 16 pigs born in total, and 26 sows had a litter with more than 16 pigs born in total. Across all litter sizes born, there was a negative relationship between litter size (total born) and litter average birth weight ($y = -0.0394x + 1.9348$, $R^2 = 0.2327$, P<0.001, Figure 4-2a). Average placental weight was not significantly related to litter size but was positively related to average litter birth weight ($y = 0.1229x + 0.0777$, $R^2=0.2219$, P<0.001, Figure 4-3).

Within the 148 sows with litters between 9 and 16 total born, 42 sows fell within the LBW, 82 within the MBW and 24 within the HBW category (Figure 4-2b). Within this range of litter size born, number of total pigs born, born alive, stillborn and born mummified were similar among birth weight categories (Table 4-5). Number of stillborns and pigs born alive as percentage of total litter size was also not different between birth weight categories (Table 4-5). Average placental weight was lower in LBW litters than in MBW and HBW litters (P<0.01; Table 4-5).

Significant positive relationships between individual birth weight of necropsied pigs and weight of the brain, liver, small intestine and *Semitendinosus* muscle (P<0.001 for all

relationships) are shown in Figure 4-4. Of the 358 piglets that were necropsied in the summer and winter of 2009, 253 piglets were from litters between 9 and 16 total born. Of these, 148 were considered true stillborns (lungs not floating) and 120 of these pigs had an individual birth weight within 0.5 kg of their litter average birth weight. These 120 piglets came from 26 LBW, 51 MBW and 13 HBW litters. A single pig from a LBW litter for which the brain weight data was missing, was removed from the analysis. Individual birth weight and weights of brain, liver, small intestine and *Semitendinosus* muscle were smaller ($P < 0.01$) in LBW than HBW litters, with MBW litters having intermediate results (Table 4-6). Moreover, LBW litters had higher brain:liver, brain:intestine and brain:muscle weight ratios than MBW and HBW litters ($P < 0.01$).

4.3.3 Repeatability of litter average birth weight within sows

Correlation analysis between litter average birth weight of three consecutive farrowings within sows established a correlation ($P<0.001$) between litter average birth weight of the first and second farrowing ($r=0.39$), between the second and third farrowing ($r=0.46$) and between the first and third farrowing ($r=0.30$). The correlation coefficient of the first two farrowings together versus the third farrowing was 0.47 ($P<0.001$). The percent of sows in LBW, MBW and HBW categories in two consecutive farrowings is given in Figure 4-5 and indicates that very few sows switched between the LBW and HBW categories in consecutive farrowings.

4.3.4 Growth performance data

Average daily gain during lactation tended to be higher ($P=0.06$) in HBW (0.23 ± 0.01 kg/day, n=24) than LBW (0.21 ± 0.01 kg/day, n=37) litters, resulting in a higher weaning weight ($P<0.001$) for HBW (6.49 ± 0.10 kg) than LBW (5.56 ± 0.08 kg) litters. Mortality rate during lactation was higher ($P<0.001$) in LBW than HBW litters (16.4% and 6.7% for LBW and HBW, respectively).

Body weight was lower ($P<0.01$) in LBW than MBW and HBW litters throughout the nursery and grow-finish periods (Figure 4-6a and 4-6b). Body weight of HBW pigs was also higher ($P=0.05$) than MBW pigs during most of the nursery and grow-finish phase (Figure 4-6a and 4-6b). By design, slaughter weight was similar between birth weight categories and within-pen variation in body weight at slaughter was also similar between birth weight categories, both when analyzed using the standard deviation (12.69, 12.35

and 12.42 kg for LBW, MBW and HBW litters, respectively: P=0.83) or the CV (5.08, 4.93 and 4.94 for LBW, MBW and HBW litters, respectively: P=0.78) as the measure of variation.

Average daily gain (**ADG**) was higher (P<0.05) in HBW than LBW and MBW litters throughout the nursery and grow-finish phase, and was higher (P<0.05) in MBW litters than LBW litters in the nursery, but similar to LBW litters in the grow-finish phase (Figure 4-7).

From one week after weaning until slaughter, average daily feed intake (ADFI) was higher (P<0.001) in HBW than LBW litters (Figure 4-8). Feed utilization efficiency (pen feed /pen weight gain) in the nursery phase tended to be higher (P=0.06) for LBW than MBW litters, but was not different from HBW litters (1.41, 1.45 and 1.42 for LBW, MBW and HBW litters, respectively). In the grow-finish phase, feed utilization efficiency was higher (P<0.001) in LBW than MBW and HBW litters (2.34, 2.40 and 2.42 for LBW, MBW and HBW litters, respectively).

Scour scores in the nursery (1 = no scours, 2 = mild scours, 3 = severe scours) was similar between birth weight categories (average score was 1.12 for LBW and 1.13 for MBW and HBW litters; P=0.90).

The number of pigs slaughtered per pen was similar between birth weight categories, meaning that the number of pigs that were taken off trial from weaning until slaughter due to mortality (2.7%, 2.3% and 2.6% for LBW, MBW and HBW, respectively) or morbidity/too slow growth (9.2%, 7.9% and 6.4% for LBW, MBW and HBW, respectively) were similar between birth weight categories.

Body weight and ADG were determined on individual pigs from LBW and HBW litters, selected to have an individual birth weight between 1.4 and 1.6 kg (n=45 for LBW and n=25 for HBW). Individual birth weight was similar between birth weight categories in these selected subgroups, but weaning weight was higher for LBW than HBW pigs (Table 4-7; P<0.05). Market weight and age at market were also higher in LBW than HBW pigs, but expected market weight at a fixed age of 166 days was similar between LBW and HBW pigs (116.7 kg vs. 113.5 kg for LBW and HBW respectively). ADG during lactation was higher in LBW than HBW pigs, but ADG from weaning until slaughter was similar between birth weight categories, resulting in a similar ADG overall from birth until slaughter (Table 4-7).

4.3.5 Carcass data

Live body weight and hot carcass weight were similar between birth weight categories, whereas age at slaughter was different ($P<0.001$) in LBW and HBW litters (Table 4-8). Loin depth, fat depth, lean meat percentage, yield percentage, grade premium and sort loss were not affected by birth weight category (Table 4-8).

4.4 Discussion

Low birth weight poses a problem for the swine industry due to its effects on postnatal survival, growth performance and carcass quality (Quiniou *et al*., 2002; Rehfeldt and Kuhn, 2006; Fix *et al*., 2010). Low birth weight can be due to factors affecting individual conceptuses within a normal litter, like inadequate provision of amniotic and allantoic fluid nutrients, disturbances in fetal metabolic and homeostatic mechanisms, and insufficiency or dysfunction of the placenta (Wu *et al*., 2006), all of which result in IUGR. Other factors causing IUGR are of maternal origin, with inadequate maternal nutrition and insufficient uterine capacity as the two major factors impairing fetal growth (Wu *et al*., 2006). It has also been suggested that IUGR can be a litter characteristic driven by an environmental effect of the dam and involving insufficient uterine capacity and IUC caused by high ovulation rates and good to moderate early embryonic survival to day 30 of gestation in higher parity sows (Foxcroft *et al*., 2006). IUC results in lower placental weight at day 30 of gestation and a lack of compensatory placental growth or development after day 30 results in smaller placental and fetal weights at day 90 (Town *et al*., 2004; Foxcroft *et al*., 2006). Consequently, all surviving conceptuses are impacted by IUGR, creating a predictable low birth weight litter phenotype (Foxcroft et al., 2009). Indeed, Bérard *et al*. (2010), who used a unilateral-hysterectomy-ovariectomy model to induce IUC, remarked that high birth weight pigs were often not available in IUC litters. This suggests that the birth weight of each pig in the litter is decreased due to IUC, resulting in more pigs with a low individual birth weight in litters experiencing IUC.

In the current trial, there was a decline in litter average birth weight of 39 g for each additional pig born across the whole population of litters recorded, consistent with earlier studies (35 g in Quiniou *et al*., 2002; 43 g in Beaulieu *et al*., 2010a). For litters of 9 to 16 total pigs born, a decline of only 26 g for each additional pig born was seen. Moreover, litter size in this subgroup only accounted for 4% of the variation in litter average birth

weight, yet litter average birth weight among the same sized litters differed by almost 1 kg, suggesting that factors other than numbers born are affecting litter average birth weight. The hypothesis that early IUC results in IUGR and fetal programming of the entire litter in more mature sows in relatively prolific damlines (Foxcroft *et al*., 2007 and 2009) would be consistent with the results of the present study. Town *et al*. (2004) studied the effects of relative IUC (15.1 vs. 9.3 embryos *in utero* at day 30 of gestation) and found that placental weight was lower at both day 30 and day 90 of gestation in control sows compared to sows with relatively un-crowded uteri: In contrast, embryo weight at day 30 was similar and effects of crowding on fetal weight were only established at day 90. Patterson *et al*. (2008) found similar results, showing a negative correlation between the number of embryos at day 30 of gestation and the number of fetuses at day 50 of gestation, and placental weight. Our observation of a lower placental weight in LBW compared to MBW and HBW litters, and a correlation across litters between average placental weight and average litter birth weight but not numbers born, is consistent with the hypothesis that the LBW litters were subjected to IUC early in gestation and persistent effects on placental development.

Another characteristic of IUGR is the 'brain-sparing effect', as reported between litters by Town *et al*. (2004) and within litters by Alvarenga *et al*. (2013). Consistent with these earlier studies, we established overall effects of litter birth weight classification on brain:organ weight ratios. Interestingly, Bérard *et al*. (2010) showed that across the range of IUC established in their UHO model, the individual birth weight of piglets exerted the most important effect on organ weights and brain:liver weight ratios. Thus all pigs with a low individual birth weight are expected to show the characteristics of IUGR, regardless of whether the low birth weight was caused by IUC of the whole litter or by other factors. However, the problem with IUC as a litter characteristic in early gestation is that most piglets in the litter are affected, whereas in more "normal" HBW and MBW litters, only a few piglets may experience extreme IUGR.

Our study showed clear effects of birth weight on testicular development. Both germ and somatic cell populations are proportional to testicular size, which means that lighter testes have lower numbers of these testicular components (germ cells, Leydig cells, Sertoli cells) compared to heavier testes. Interestingly, the interstitial cells seen in the high birth weight males seem to be replaced by adipose cells in the low birth weight males, as can

be observed in Figure 4-1. It has been demonstrated that Sertoli cells provide the environment that protects and nourishes germ cells and supports their development to viable sperm (França & Chiarini-Garcia, 2005) and that after proliferation during the pre-pubertal period, the total number of Sertoli cells achieved will determine testicle size in adulthood, as well as the sperm production capacity (Cooke *et al.*, 1992; Hess *et al.*, 1993). In the context of the multiplication level of sire-line genetic programs, our results have important implications for the effects of litter birth weight phenotype on the lifetime sperm production and libido of prospective AI boars. If mature sows in these sire-line programs show the same repeatability in litter birth weight phenotype as in the present study, selection of potential AI boars from high birth weight litters would be predictive of better lifetime productivity in the boar stud. Limitations due to initial birth weight phenotype would also be consistent with the recent reports of beneficial effects of improved pre-weaning growth of prospective AI boars achieved by rearing these boars in smaller litters during lactation (Flowers, 2008).

The higher pre-weaning mortality in LBW litters is also in agreement with earlier findings (Smit, 2007). As both mortality and morbidity, as measured by scour scores in the nursery and the number of pigs taken off trial due to disease or slow growth in the nursery and grower-finisher, were similar between birth weight categories, targeting management interventions in the farrowing house to reduce the impact of low litter birth weight should be seen as a high priority, particularly given the repeatability of the low birth weight phenotype.

Another important outcome of IUGR is reprogramming of myogenesis (Rehfeldt and Kuhn, 2006). The brain:*Semitendinosus* muscle weight ratio was higher in LBW litters, as also reported by Town *et al*. (2004) in day 90 fetuses from relatively crowded uteri. Lower muscle weight and a higher brain:muscle weight ratio at birth in pigs with low birth weight has also been shown before (Tristán *et al*., 2009; Alvarenga *et al*., 2013), showing the reprogramming effects of IUGR on myogenesis. Moreover, Bérard *et al*. (2010) showed that, although IUC had no effect on the weight of the *Semitendinosus* muscle, it did decrease the number of secondary myofibers (Bérard *et al*., 2010). The fact that they did not find an interaction between individual birth weight and treatment (IUC vs. control) means that IUC effects on muscle development were similar for all individual birth weights selected (Bérard *et al*., 2010). The number of muscle fibers at birth is

important because there is no increase in muscle fiber numbers after birth (Rehfeldt and Kuhn, 2006) and growth after birth occurs through hypertrophy of existing muscle fibers. Because Bérard *et al*. (2010) found lower muscle fiber numbers in litters experiencing IUC, and because our assumption is that LBW litters had experienced IUC, it was expected that pigs in LBW litters would show lower body weight and average daily gain (ADG) than pigs in HBW litters. Indeed, LBW litters had lower body weights at all times, and the difference in body weight between LBW and HBW litters increased over time due to lower ADG in LBW than HBW litters during lactation (tendency), and the nursery and grow-finish phases. This resulted in LBW litters needing 9 more days to reach the same market weight as HBW litters and suggests that segregated management of low birth weight progeny would allow more efficient use of barn turns at the grow-finish stages of production.

However, when looking at individual piglets with a birth weight between 1.4 and 1.6 kg, piglets from LBW litters had a higher ADG during lactation than piglets from HBW litters, which was not expected. It is possible that competition between littermates for milk resources played a role in this result; pigs with a birth weight between 1.4 and 1.6 kg were the biggest pigs in LBW litters, but were the smaller pigs in HBW litters. It is known that the biggest pigs in a litter generally have access to the best teats, and therefore secure a better supply of colostrum and milk, whereas the smaller pigs in a litter cannot compete effectively with their bigger litter mates (Devillers *et al*., 2007). Once the pigs were placed in nursery pens, this advantage in size diminished, and the ADG was similar between LBW and HBW pigs between 1.4 and 1.6 kg birth weight. The fact that ADG was similar after weaning for this subgroup apparently conflicts with our hypothesis of slower growth as a characteristic of entire LBW litters. However, it is still possible that LBW pigs end up with more fat and less lean tissue when slaughtered at the same body weight as HBW pigs, due to the lower numbers of muscle fibers in low birth weight pigs (Rehfeldt and Kuhn, 2006; Tristán *et al*., 2010; Bérard *et al*., 2010).

The two concepts of lean growth are, 1) it increases after 20 kg live weight, reaches a plateau, and declines thererafter, and 2) as feed intake increases, a linear response in lean growth and fat accretion occurs (Schinckel, 1997). Schinckel *et al*. (2004) showed that the piglets in the lightest twentieth percentile at birth continued to deviate from the weight of the other pigs with increasing time after weaning, due to lower ADG. Based on the concepts of lean growth and muscle fiber growth, it is expected that low birth weight

pigs have faster fiber growth and reach the plateau of lean growth earlier than high birth weight pigs (Rehfeldt and Kuhn, 2006). After the plateau of fiber growth has been reached, nutritional energy is mainly used to store fat in the body (Schinckel, 1997). Indeed, it was shown that low birth weight pigs have the largest fibers at slaughter, and that they tended to have lower meat percentage and lower loin muscle area, while having higher internal fat percentage (Rehfeldt and Kuhn, 2006). However, carcass weight at a fixed age was also different between birth weight categories in that research, which makes it harder to directly compare carcass traits between birth weight categories. For this reason, in the current trial pigs were slaughtered by pen at a fixed end weight. Unfortunately, some of the information for the HBW pens was lost due to problems in the slaughterhouse. The number of pens left was insufficient to show differences between birth weight categories for any of the carcass traits, including fat depth and lean tissue yield. However, fat depth was numerically higher in LBW than HBW litters. Gondret *et al*. (2006) also found higher backfat thickness in pigs with a low individual birth weight compared to littermates with a high individual birth weight, and Bee (2004) found higher adipose tissue yield in low vs. high birth weight pigs. However, others have not shown differences in backfat or adipose tissue yield between pigs with low vs. high birth weight when slaughtered at the same weight (Wolter *et al*., 2002; Gondret *et al*., 2005). Results for lean meat percentage are also inconsistent; Gondret *et al*. (2006) found lower lean meat percentage in low than high birth weight pigs, while Wolter *et al*. (2002), Bee (2004) and Gondret *et al*. (2005) did not show any differences in lean meat percentage between birth weight categories. Further research is needed to investigate the effect of low birth weight on carcass composition traits.

A big problem in all-in all-out systems is the huge variation within pens in body weight at time of slaughter. Although the common practice is to sort pigs by size at entry of the nursery and/or grow-finish barn (Deen, 1997; Tokach, 2004), research has shown that this is not effective in decreasing weight variation (O'Quinn *et al*., 2001), while it increases aggressive behaviour during the 2-day post regrouping period (O'Connell *et al*., 2005). Schinckel *et al*. (2004) showed that pigs in the smallest twentieth percentile at birth grow slower after weaning and are responsible for the majority of variation in pig weights after weaning. Pigs sourced from our LBW litters are likely to overlap to a great extent with the overall twentieth percentile of lowest birth weight pigs in all litters born. Given this overlap, an option raised by the current trial is to sort nursery and grow-finish

pigs by litter average birth weight rather than by individual body weight. Indeed, a CV of approximately 5% for within-pen weight variation around slaughter as reported in our trial was smaller than is generally observed. Literature gives CV's anywhere between 6 to 8% (O'Quinn *et al*., 2001) which was considered low by the authors, and between 8 and 14% (Dedecker, 2002; O'Connell *et al*., 2005).

Another management option for the nursery and grow-finish phase is segregated management of the different litter birth weight phenotypes. Segregated management allows for different feeding strategies. Beaulieu *et al*. (2010b) showed that pigs with lighter birth weights showed a greater positive response to a complex diet after weaning than heavier birth weight pigs and concluded that the Phase 1 diet in the nursery could be used more efficiently and cost-effectively when targeted specifically to the low birth weight pigs at weaning. Moreover, it has been shown that low birth weight pigs have a lower feed efficiency than high birth weight pigs (O'Quinn *et al*., 2001; Schinckel *et al*., 2010). This could be due to the effects of piglet birth weight on intestinal morphology as reported by D'Inca *et al*. (2011) and Alvarenga *et al*. (2013). Although reduced feed utilization efficiency in LBW litters might have been expected in our study, feed utilization efficiency tended to be higher for LBW compared to MBW litters in the nursery phase and was significantly higher in the grow-finish phase than feed utilization efficiency of MBW and HBW litters. It is not clear why LBW litters had a higher feed efficiency. Nonetheless, it is clear that pigs from low birth weight litters have different nutritional needs than those of high birth weight litters, and segregated management would help to optimize the feeding of both populations.
Another potential advantage of segregated management would be that low birth weight litters could be marketed differently from the rest of the population. Because low birth weight pigs likely reach the plateau of lean growth earlier than high birth weight pigs (Rehfeldt and Kuhn, 2006), LBW litters should either be marketed at a lower slaughter weight, or sent to a market demanding higher fat percentages.

In conclusion, litters with low average birth weight showed the benchmarks for intra-uterine growth retardation, like lower placental weight and the brain-sparing effect. Litter average birth weight was relatively repeatable within sows. Low birth weight may affect testicular size and the germ and somatic cells population in the neonatal piglet. Moreover,

low birth weight litters grew slower during all phases of production and needed 9 more days to reach the same market weight as pigs from high birth weight litters.

Table 4-1. Calculated nutritional composition of the corn/soybean meal based gestation and lactation diets consumed by all sows on trial

	Gestation	Lactation
Digestible lysine, %	0.59	0.94
ME, MJ/kg	13.28	13.32
Ca, %	0.91	0.95
Available P, %	0.57	0.59
Vitamin A, KIU/kg	11.33	11.31
Vitamin D, KIU/kg	2.20	2.20
Vitamin E, IU/kg	128.04	127.23
Vitamin K, mg/kg	1.43	1.43

Table 4-2. Cut-off weights used to classify litters as low (LBW) or high (HBW) birth weight for different litter sizes born, relative to the mean and standard deviation (St.Dev.) of all litters born in the previous two farrowings from the same sows

Litter size	Mean (kg)	St.Dev. (kg)	LBW [1] (kg)	HBW [2] (kg)
9	1.57	0.23	< 1.34	> 1.80
10	1.63	0.29	< 1.34	> 1.92
11	1.54	0.24	< 1.30	> 1.78
12	1.52	0.21	< 1.31	> 1.73
13	1.50	0.22	< 1.28	> 1.72
14	1.42	0.20	< 1.22	> 1.62
15	1.40	0.20	< 1.20	> 1.60
16	1.42	0.16	< 1.26	> 1.58

[1]LBW was defined as litters being more than one standard deviation below the litter size mean based on data of the preceding 2 farrowings of the sows on trial.

[2]HBW was defined as litters being more than one standard deviation above the litter size mean based on the same data.

Table 4-3. Calculated chemical composition of the nursery and grow-finish diets fed to all pigs on trial

	Days fed	Digestible lysine (%)	ME (MJ/kg)
Nursery			
Phase 1	7	1.45	14.99
Phase 2	7	1.40	14.03
Phase 3	7	1.38	14.23
Phase 4	21	1.28	14.77
Grow-Finish			
Phase 1	21	1.23	14.30
Phase 2	21	1.05	14.30
Phase 3	21	0.91	14.30
Phase 4	21	0.80	14.31
Phase 5	Until slaughter	0.73	14.30

Table 4-4. Biometrical and histomorphometrical data of the testes from high (HW) and low (LW) birth weight piglets, born in high or low average birth weight litters respectively

	HW	LW	RSD	P-value
Biometrical data				
n	22	18		
Castration body weight (kg)	2.96	1.90	0.43	< 0.01
Testicular weight (g)	0.76	0.49	0.28	< 0.01
Gonadosomatic Index (GSI) [a]	0.026	0.025	0.008	NS
Histomorphometrical data				
n	5	5		
Testicular weight (g)	1.04	0.38	0.24	<0.01
Absolute numbers (x 10^6)				
Sertoli Cells	0.13	0.05	0.22	< 0.05
Germ cells	0.03	0.02	0.01	= 0.056
Leydig Cells	0.94	0.42	0.21	< 0.01
Number/gram of testes (x10^6)				
Sertoli Cells	0.12	0.14	0.01	NS
Germ cells	0.03	0.04	0.01	NS
Leydig Cells	0.94	1.08	0.08	NS
Correlations				
Testicular weight x Body weight	r = 0.56			< 0.01
Testicular wt x Sertoli cell number	r = 0.93			< 0.01
Body weight x Sertoli cell number	r = 0.76			< 0.05

Data are the LSMeans, RSD = residual standard deviation

[a] Gonadosomatic Index (GSI) = Testicular weight / Body weight x 100

Table 4-5. Characteristics at birth for litters between 9 and 16 total piglets born for low (LBW), medium (MBW) and high (HBW) birth weight litters

	LBW	MBW	HBW	RSD	P-value
n	42	82	24		
Total born	12.7	12.9	13.5	2.0	0.30
Born alive	11.3	11.7	12.5	2.2	0.14
Born alive (% of total born)	89.5	91.2	93.2	10.3	0.38
Stillborn	1.3	1.2	0.9	1.4	0.55
Stillborn (% of total born)	10.5	8.8	6.8	10.3	0.38
Mummies	0.4	0.4	0.1	0.8	0.35
Litter ave bw (kg) [a]	1.12 [A]	1.45 [B]	1.79 [C]	0.11	<0.001
Total litter bw (kg) [a]	14.12 [A]	18.58 [B]	23.68 [C]	2.43	<0.001
Ave placental wt (kg) [b]	0.21 [A] (n=16)	0.26 [B] (n=48)	0.28 [B] (n=10)	0.06	0.01
Litter ave bw of selected litters (kg) [c]	1.13 (n=38)	1.42 (n=64)	1.75 (n=30)	0.11	<0.001

Data are the LSMeans, RSD=Residual standard deviation, ave = average, bw = birth weight

[A,B, C] LSMeans in a row with different superscripts are significantly different at P<0.05

[a] Total number of pigs born in litter used as covariate

[b] Only taking into account litters where more than 50% of the placentae were recovered

[c] Litters of the different birth weight categories were randomly selected to be followed in the nursery and grow-finish phases

Table 4-6. Data of necropsied piglets for litters with low (LBW), medium (MBW) or high (HBW) average litter birth weight

	LBW	MBW	HBW	RSD	P-value
n	25	51	13		
Individual birth wt (kg)	1.03 [a]	1.41 [b]	1.84 [c]	0.25	<0.001
Brain wt (g)	28.74 [a]	29.48 [a]	31.42 [b]	2.54	0.01
Liver wt (g)	36.82 [a]	48.02 [b]	56.53 [c]	12.70	<0.001
Small intestine wt (g)	34.38 [a]	49.72 [b]	56.60 [b]	11.51	<0.001
Muscle wt (g)	1.90 [a]	2.39 [b]	3.02 [c]	0.67	<0.001
Brain:liver wt ratio	0.83 [a]	0.66 [b]	0.63 [b]	0.20	0.001
Brain:intestine wt ratio	0.88 [a]	0.63 [b]	0.57 [b]	0.17	<0.001
Brain:muscle wt ratio	16.24 [a]	13.50 [b]	11.38 [b]	4.04	<0.01

Data are the LSMeans, RSD = Residual standard deviation, wt = weight

[a, b, c] LSMeans in a row with different superscripts are significantly different at P<0.05

Table 4-7. Body weight and average daily gain (ADG) for pigs from low (LBW) and high (HBW) birth weight litters with an individual birth weight between 1.4 and 1.6 kg

	LBW	HBW	RSD	P-value
n	45	25		
Individual bw (kg)	1.49	1.51	0.06	0.15
Wean Wt (kg)	6.38	5.83	0.88	<0.05
Market Wt (kg)	119.2	109.2	10.7	<0.001
Age at slaughter (days)	170.6	161.4	2.6	<0.001
ADG lactation (g)	248.4	218.4	1.4	<0.01
ADG Wean-Finish (g)	753.5	734.7	2.3	0.34
ADG total (g)	694.9	671.3	2.0	0.17

Table 4-8. Carcass data for low birth weight (LBW), medium birth weight (MBW) and high birth weight (HBW) litters

Carcass data	LBW	MBW	HBW	RSD	P-value
n	9	15	5		
Age at slaughter (days)	174.7 [a]	170.9 [b]	165.6 [c]	2.7	<0.001
Live weight (kg)	115.8	116.0	116.5	1.7	0.79
Hot carcass weight (kg)	88.2	88.4	88.0	1.3	0.84
Loin depth (mm)	71.05	71.61	70.22	1.68	0.38
Fat depth (mm)	15.38	15.61	14.55	1.13	0.31
Lean meat (%)	56.4	56.4	56.5	0.4	0.93
Yield (%)	76.1	76.3	75.6	0.9	0.33
Grade premium (US$)	6.15	6.16	6.22	0.26	0.90
Sort loss (US$)	-0.93	-0.83	-0.80	0.29	0.61

[a, b, c] LSMeans in a row with different superscripts are significantly different at P<0.05

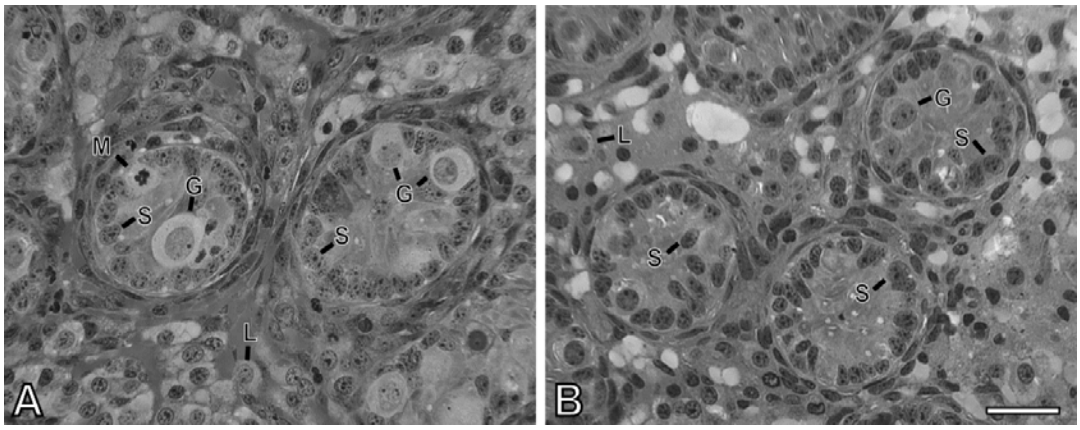
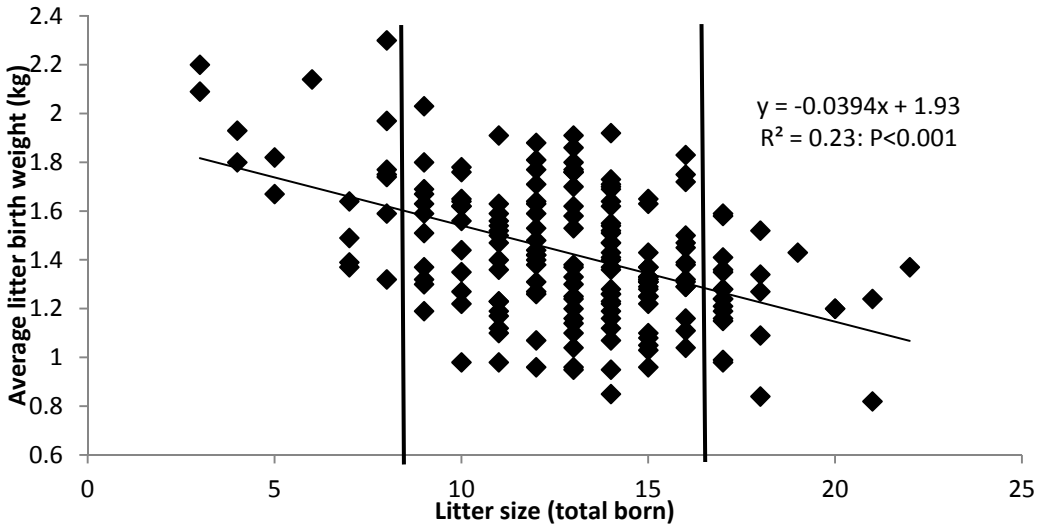


Figure 4-1. Photomicrographs of transversal sections of testicular cords from 6-d old piglets of high (A) and low (B) birth weights. Observe the germ cells (G), the nuclei of the Sertoli cells (S), Leydig cells (L), and presence of cell division (M: mitosis). Toluidine blue-sodium borate staining. Bar represents: 30 µm.

a.



b.

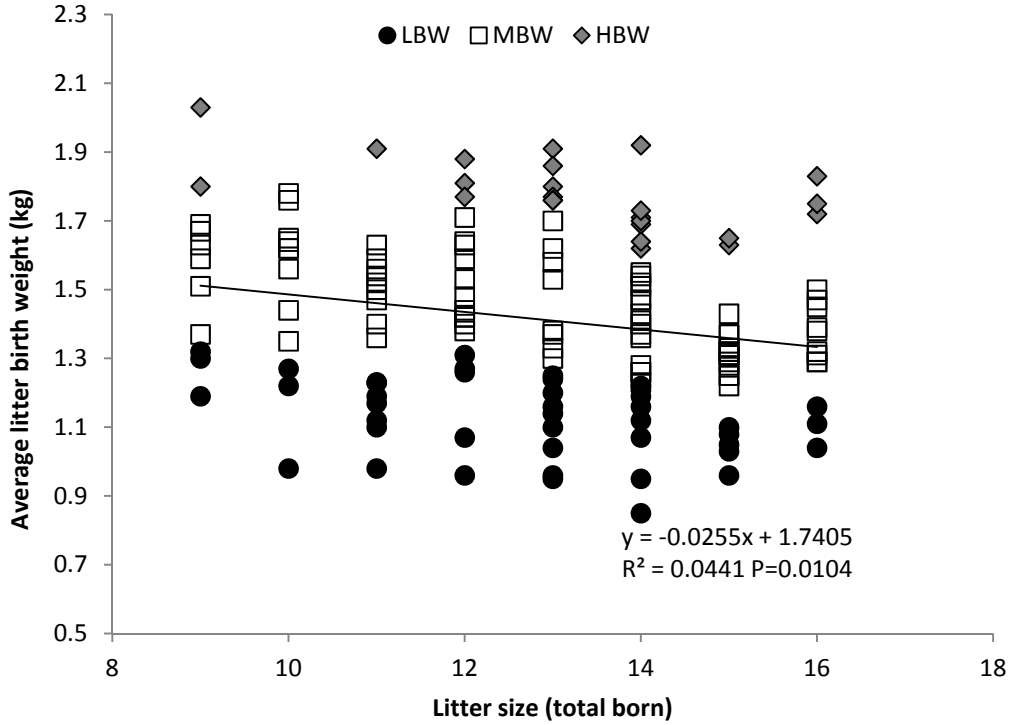


Figure 4-2. Relationship between litter size (total pigs born) versus average litter birth weight for a) all litter sizes (n=192) and b) litters between 9 and 16 total born (n=148). The litters between 9 and 16 total born were classified as low (LBW), medium (MBW) or high (HBW) average birth weight.

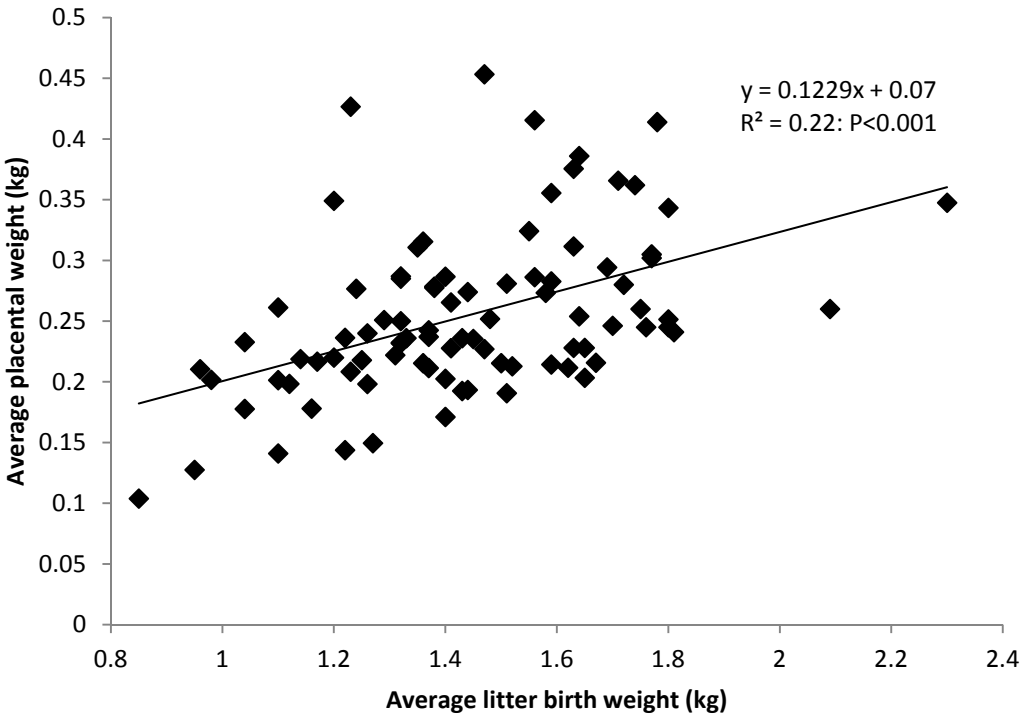


Figure 4-3. Relationship between average litter birth weight and average placental weight per litter (n=89)

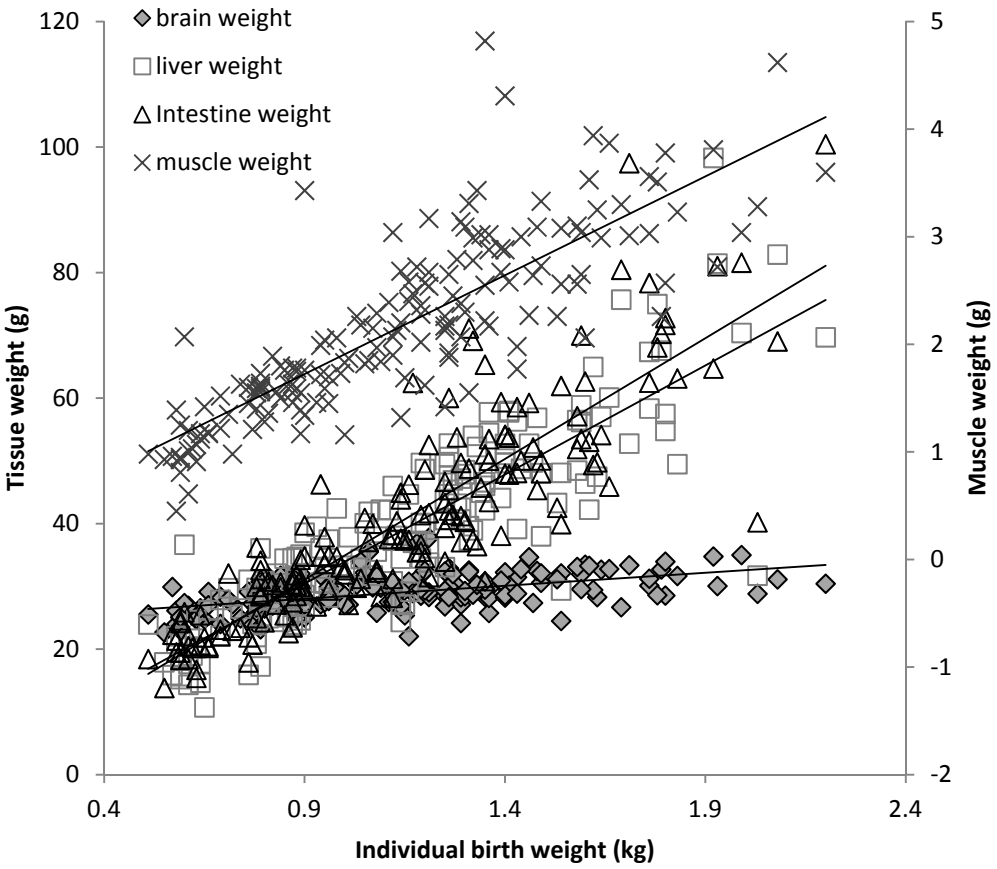


Figure 4-4. Relationship between individual birth weight and wet weight of brain, liver, small intestine and *Semitendinosus* muscle (n=169). Regression lines:

Brain: $y = 4.15x + 24.28$, $R^2 = 0.29$, $P<0.001$

Liver: $y=34.78x – 0.87$, $R^2 =0.75$, $P<0.001$

Small intestine: $y = 38.48x – 3.57$, $R^2 =0.77$, $P<0.001$

Semitendinosus muscle: $y = 1.84x + 0.07$, $R^2 =0.68$, $P<0.001$

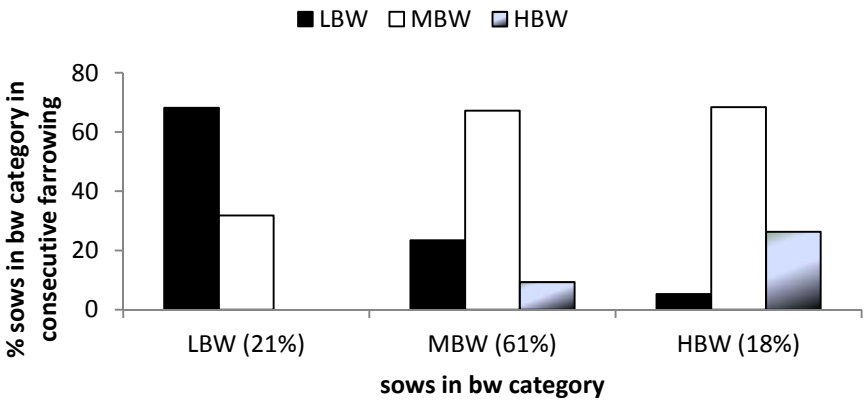
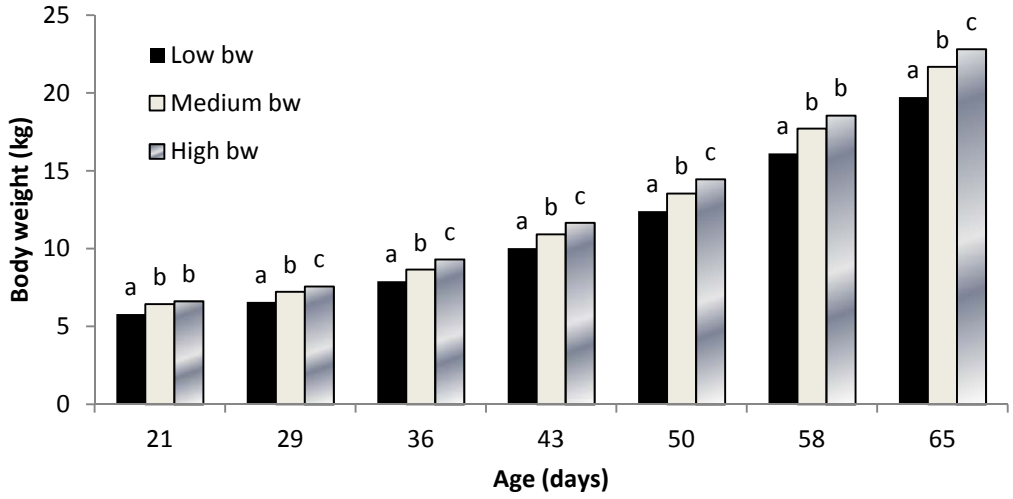


Figure 4-5. Classification of sows into low (LBW), medium (MBW) and high (HBW) litter average birth weight in the first farrowing (on the x-axis) and the percentage of those sows falling in the LBW, MBW or HBW category in the second farrowing (on the y-axis), showing the repeatability of litter average birth weight within sows; few sows switch between the low and high litter birth weight category (n=105).

a.



b.

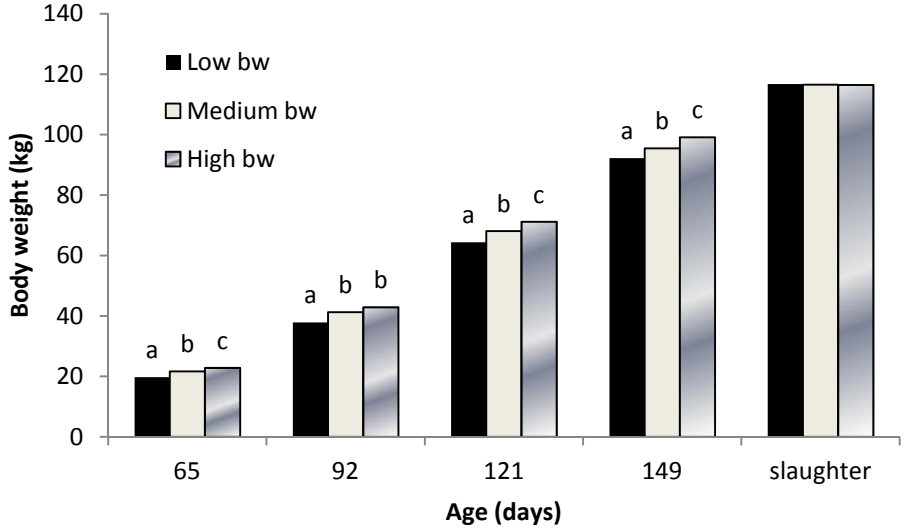


Figure 4-6. Body weight for low birth weight (bw), medium birth weight and high birth weight litters in a) the nursery phase (P-value for birth weight category: <0.001, for Time: <0.001 and birth weight category by time interaction: 0.001), and b) the grow-finish phase (P-value for birth weight category: <0.001, for Time: <0.001 and birth weight category by time interaction: <0.01).

Columns within age without common superscript are significantly different at P<0.05

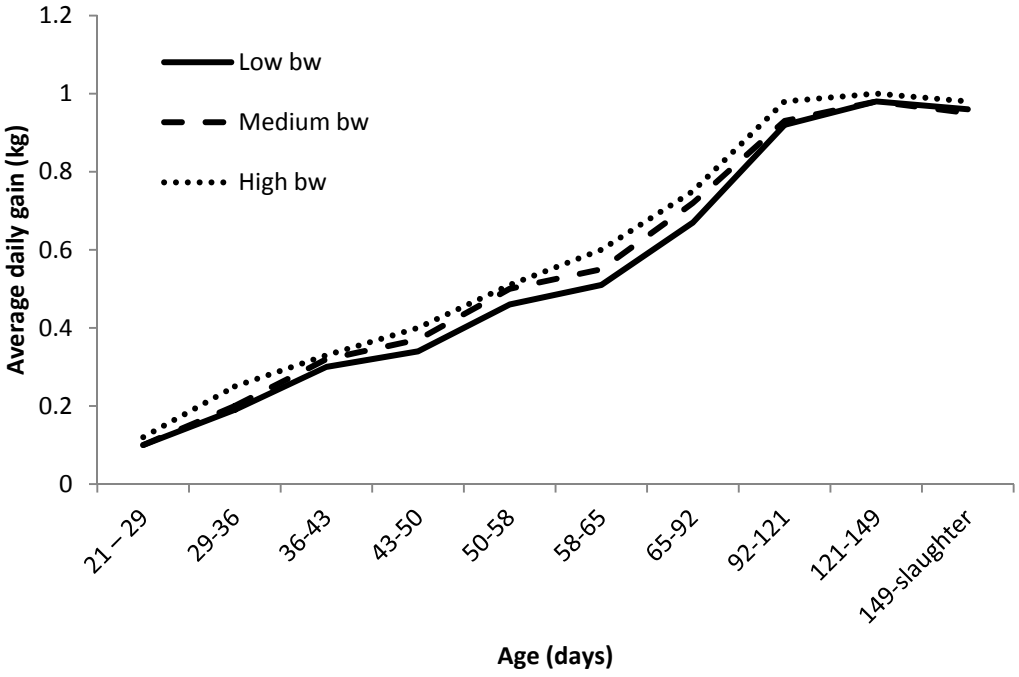


Figure 4-7. Average daily gain for low birth weight (bw), medium birth weight and high birth weight litters in the nursery (21 to 65 days of age) and the grow-finish period (65 days of age till slaughter). P-values in the nursery for birth weight category: <0.001, for Time: <0.001 and birth weight category by time interaction: 0.47. P-values in the grow-finish phase for birth weight category: <0.05, for Time <0.001 and for birth weight by time interaction: 0.28.

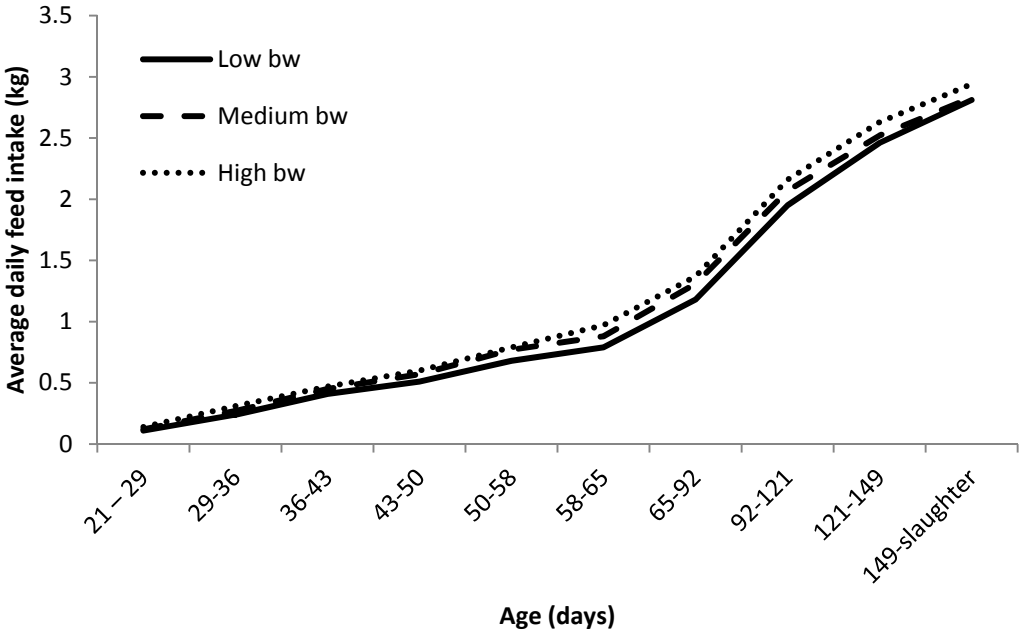


Figure 4-8. Average daily feed intake for low birth weight (bw), medium birth weight and high birth weight litters during the nursery period (21 – 65 days of age) and the grow-finish period (65 days of age until slaughter). P-values in the nursery for birth weight category: <0.001, for Time: <0.001 and for birth weight category by time interaction: <0.01. P-values in the grow-finish phase for birth weight category: <0.001, for Time: <0.001 and for birth weight category by time interaction: 0.39.

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Chapter 5: Dietary enrichment with a marine-oil based n-3 LCPUFA in sows with predicted birth weight phenotypes I: Effects on pre-weaning litter quality and growth performance

5.1 Introduction

An important factor in farm profitability is the size and growth uniformity of pigs weaned. Uniformity in body weight at time of slaughter is critical for efficient use of all-in/all-out systems (Deen, 1997). Previous research has shown that litters with a low average birth weight take 9 days longer to reach slaughter weight than litters with a high average birth weight (Chapter 4). This research has also shown that litter birth weight phenotype is quite repeatable within sows and this information could be used to plan management strategies directed at potential low birth weight litters. One such strategy is nutritional intervention in sows with a predicted low litter birth weight phenotype.

N-3 LCPUFA supplementation to sows during (parts of) gestation and lactation has been shown to increase offspring growth rate (Rooke *et al*., 2000 and 2001b; Mateo *et al*., 2009; Smit *et al*., 2012 and Chapter 3). Feeding n-3 LCPUFA only to sows with a predicted low litter birth weight phenotype might therefore help to reduce the gap in growth rate between low and high birth weight litters, which would ultimately decrease the variation in body weight at slaughter. However, before implementing such a management strategy, it is important to investigate whether marine-oil based n-3 LCPUFA enrichment of the sow diet results in the same increase in growth rate in low birth weight litters as seen when feeding the entire sow population.

This research investigates the effects of marine-oil based n-3 LCPUFA enrichment to the sow from weaning, during the rebreeding period, during gestation and until end of lactation on litter characteristics from birth until weaning. The possible interaction between marine-oil based n-3 LCPUFA treatment and litter birth weight phenotype are also investigated. It is hypothesized that low birth weight litters will benefit more from marine-oil based n-3 LCPUFA supplementation than high birth weight litters. The present paper describes the overall experimental approach used, further data on the repeatability and characteristics of low birth weight litters, and observed effects of litter birth weight and marine-oil based n-3 LCPUFA enrichment until weaning. Effects of

litter birth weight phenotype and marine-oil based n-3 LCPUFA enrichment to sows on post-weaning performance of the litters will be described and discussed in Chapter 6.

5.2 Material and Methods

5.2.1. Animals and Treatments

This study was performed according to Canadian Council on Animal Care guidelines and JBS United Inc. ethical guidelines. Multiparous Large White x Landrace terminal line sows (Camborough; PIC, Nashville, TN, USA) were managed according to approved protocols at the JBS United Inc. sow research farm (Bache Farm, IN, USA). A total of 163 sows, ranging between parity 4 and 8 (mean = 4.9 ± 0.9) that were a part of five consecutive weekly breeding groups, were rebred after weaning. Information on litter average birth weight of the preceding three litters was available for all sows and after weaning, sows were pair-matched by parity and litter average birth weight of the previous 3 litters. Within pairs (n=80), sows were allocated to be fed either standard corn/soybean meal based gestation and lactation diets (**CON**; Table 5-1), or the same diets enriched with 0.5 % of a n-3 LCPUFA rich supplement (**mLCPUFA**; Table 5-1) at the expense of corn. The n-3 LCPUFA product (Gromega Ultra 365, JBS United Inc, Sheridan, IN, USA) was the same product as used in Chapter 3; it is a marine-oil based supplement rich in eicosapentaenoic acid (**EPA**) and docosahexaenoic acid (**DHA**) which was stabilized to prevent auto-oxidation. Diets were fed from weaning, during rebreeding, throughout gestation and from farrowing until the end of a 21-day lactation. Three sows (2 mLCPUFA and 1 CON) not allocated to a pair due to uneven numbers of sows in some weaned groups, and additional sows in a pair where the pair-matched sow did not achieve pregnancy, were considered as 'incomplete pairs' in the analysis.

Sows were induced to farrow with a 2 ml injection of a prostaglandin $F_{2\alpha}$ analogue (Lutalyse, Pfizer Animal Health, New York, NY, USA) on day 114 of gestation if no signs of parturition were apparent. Within 24 hours after birth, all piglets were ear-tagged and individual birth weight of all pigs born was recorded. Litter average birth weight was calculated as total birth weight of all pigs in a litter, divided by the total number of pigs born in that litter. Each litter between 9 and 16 total pigs born was classified as low (**LBW**), medium (**MBW**) or high (**HBW**) litter average birth weight, as described previously (Chapter 4.2.1 and Table 4-2). Due to the small proportion of HBW litters, MBW and HBW were combined into one class (**MHBW**) for litter analysis. Cross-

fostering of piglets, to standardize litters suckled to 10 to 12 pigs per sow, occurred within treatment only, but irrespective of birth weight classification. Each time a piglet was removed or added to a litter, the date, piglet weight and reason for removal or addition were recorded. All pigs were again individually weighed the day before weaning.

5.2.2 Measurements before weaning

Within 24 hours after birth and before cross-fostering, sow ID, parity, date of birth, total number of piglets born, number of piglets born alive, number of stillborns, number of mummies, as well as individual birth weight and sex of all piglets born, were recorded for each litter.

Up to a maximum of two males and two female stillborn piglets and piglets that died within 12 hours after birth from any litter were dissected within 24 hours after birth. Stillbirth was confirmed by removing the lungs and conducting a "lung floatation" test to determine if the piglets were born dead and never breathed (lungs not floating) or if they were born live (lungs floated) but died soon after birth. Stillborns that were smaller than 2 standard deviations below the litter average birth weight were considered to be runts and were not dissected. The wet weights of the brain, liver, small intestine, thymus, kidney, adrenal, heart, lungs and *Semitendinosus* muscle of the right leg were recorded at necropsy, and samples of brain, liver, and muscle tissues were saved and stored at -20°C until later analysis (see Chapter 6).

The number of placentae recovered and total placental wet weight for each sow farrowed were also recorded, from which an average placental wet weight was calculated. However, placental data were only used for subsequent statistical analysis when more than 50% of the placentae in a litter were recovered, which occurred in 76 litters.

Blood samples were taken from all sows on day 113 of gestation into non-heparinized vacutainer tubes (BD, Fisher Scientific, Ottawa, ON, Canada) and held at ambient temperature until centrifugation (Jorvet J-502) at 1034 x *g*. Serum was then harvested and frozen at -20°C. Colostrum samples were obtained, without oxytocin administration, from as many sows as possible. The aim was to obtain the colostrum sample within 12 hours after farrowing of the first piglet. However, this was not always achieved, and the range of colostrum sample collection was from 10 hours before to 25 hours after

farrowing. Samples were "milked" manually from all teats, pooled within a sow, and then stored at -20°C until further analysis.

5.2.3 Immunoglobulin G measurements

IgG were assayed by ELISA in serum and whole colostrum using a pig IgG ELISA Quantitation Kit (Bethyl, Texas, USA, Ref. E100-104). The plates were coated with 100 µL of goat antipig IgG-Fc fragment diluted at 1% in 0.05 M carbonate-bicarbonate solution (pH 9.6, Sigma, St-Louis, USA). Subsequently, the plates were blocked for 30 minutes at room temperature, or overnight at 4 °C, with TBS (Fisher Scientific, Ottawa, ON, Canada) containing 1% BSA (Sigma). Serum samples were diluted 1:1.6 x 10^5 and colostrum samples were diluted 1:2.4 x 10^6 in TBS with 0.05% Tween 20 (Caledon, Georgetown, ON, Canada) and 1% BSA, added in duplicate to the plates (100 µL / well) and incubated for 1 h at room temperature. Thereafter, the plates were incubated for 1 h at room temperature with 100 µL peroxidase-labeled anti-pig IgG-Fc fragment diluted 1 : 7.5 x 10^4 in TBS with 0.05% Tween 20 and 1% BSA. Then, 100 µL of a substrate containing 3,3',5,5'-tetramethylbenzidine was added and the plate was placed in the dark. Between each step, plates were washed five times with a plate washer (Skanwasher 400, Molecular Devices, Sunnyvale, CA, USA) with TBS containing 0.05% Tween 20. The colorimetric reaction was stopped after 15 minutes with 100 µL of a 0.18 M H_2SO_4 solution (Fisher Scientific) and absorbance at 450 nm was recorded using an ELISA plate reader (Spectramax M3, Molecular devices, Sunnyvale, CA, USA). An assay sensitivity of 23 ng/ml as described by Devillers *et al*. (2004) was consistent with the upper limits of the confidence interval of the lowest point on the curve in the current trial. Dilution curves of serum (1 : 20 000 to 1 : 320 000) and colostrum (1 : 100 000 to 1 : 3 200 000) were parallel to standard curves. The intra-assay coefficient of variation was calculated as the mean CV of 20 samples within each assay and was 12.3% for serum and 18.5% for colostrum. Inter-assay CV could not be calculated due to issues with stability of samples used as internal standards after thawing and refreezing, but each assay contained similar numbers of CON and mLCPUFA samples. Only samples that were run for the first time and that had duplicates with a CV < 20% were used in the final analyses, which resulted in 67 serum samples and 99 colostrum samples (not necessarily from the same sows) being used for analysis. Two serum samples with biologically unrealistic high values of IgG concentration were considered outliers and also removed from the analysis.

5.2.5 Statistical analysis

All variables were tested for normality prior to analyses, using the univariate procedure in SAS. Also the homogeneity of variance of the residuals was tested for each variable, using the Bartlett and Levine's tests in SAS. When variables were not normally distributed, they were only transformed if the variance of residuals was not homogeneous and if it improved the fit of the model. None of the variables needed transformation on this basis.

Data were analyzed using the MIXED procedure of SAS (SAS Institute, Cary, NC, USA) as a randomized incomplete block design, with blocks based on sow pairs. If one sow of a pair was not pregnant or was taken off trial at any other point in time, the other sow of the pair remained on trial and was considered an incomplete block. The model included sow treatment (CON or mLCPUFA) as a fixed effect and pair as a random effect. Sow was used as the experimental unit for all parameters before weaning, including treatment effects on litter growth, and all individual measurements of piglets before weaning were averaged within a litter (sow) before statistical analysis. Data from necropsied pigs were analyzed as a nested design, with pig-within-sow as the experimental unit, and treatment and sex as fixed effects. Associations between individual birth weight and tissue weights of necropsied pigs were analyzed using regression analysis. Analysis of a subset of litters (with 9 to 16 total pigs born) had both sow treatment and litter birth weight classification (LBW or MHBW) as fixed effects and pair as a random effect. Categorical data like farrowing rate and pre-weaning mortality was analyzed separately using the generalized logit function (proc CATMOD in SAS). Correlation analysis was performed using the CORR procedure in SAS.

Data presented here are given as least square means, unless otherwise stated, and data in the figures as means. Probability values < 0.05 were considered significant and values < 0.10 were used to describe trends.

5.3 Results

5.3.1 Birth to weaning data for the entire dataset

Of the sows weaned, 85.9% were bred successfully and 95.7% of these bred sows farrowed (64 CON and 70 mLCPUFA sows), including 53 complete sow pairs. Breeding

rate and farrowing rate were similar for CON and mLCPUFA sows. The total number of pigs born and born alive were lower (P=0.01) in mLCPUFA than CON sows, while the number of stillborn and mummified pigs were similar between treatments (Table 5-2). Number of stillborns and pigs born alive as a percentage of total litter size was not different between treatments (Table 5-2). The negative relationship between litter size (total born) and litter average birth weight was similar for CON and mLCPUFA litters (Figure 5-1). Average placental weight was not related to litter size, was positively related to litter average birth weight for both CON and mLCPUFA litters (Figure 5-2), and was lower (P<0.05) in mLCPUFA than CON sows (Table 5-2).

Necropsied piglets had similar average individual birth weights for both treatments and both sexes. Treatment did not have a significant effect on any of the tissue weights or brain:tissue weight ratios (Table 5-3). Lung weight was lower in male than female pigs, resulting in a higher brain:lung weigh ratio for males (Table 5-3) and the brain:kidney weight ratio was also higher in males than females. All other tissues and brain:tissue weight ratios were similar between sexes (Table 5-3).

After cross-fostering within treatment, the litter size at day 1 and day 20 of lactation was again higher (P<0.001) in CON than mLCPUFA litters (Table 5-4). Average and total litter weight at day 1 was similar between treatments, but average and total litter weight at day 20 was higher (P<0.05) in mLCPUFA than CON litter, due to a higher (P<0.05) average daily gain (ADG) for mLCPUFA than CON litters (Table 5-4). Pre-weaning mortality (17.0 % and 18.2 % for CON and mLCPUFA, respectively) was similar between treatments.

5.3.2 Birth to weaning data for litters of 9 to 16 total born

The total number of pigs born and the number born alive was lower (P<0.01 and P<0.05, respectively) in mLCPUFA than CON sows, while they were not different between LBW and MHBW birth weight categories (Table 5-5). The number of stillborns and mummies were similar between treatments, but the number of stillborns tended to be higher (P=0.07) in LBW than MHBW litters and the number of mummies was higher (P<0.01) in LBW than MHBW litters. By design, litter average birth weight was lower (P<0.001) in LBW than MHBW litters, but was not different between mLCPUFA and CON sows (Table 5-5). Average placental weight was lower (P=0.01) in LBW than MHBW litters, but was not different between treatments (Table 5-5). Results for treatment and sex

effects on tissue weights and brain:tissue weight ratios for necropsied pigs born in litters of 9 to 16 total born were similar to those of the entire dataset (Table 5-6). Individual birth weight and tissue weights of necropsied pigs were higher ($P<0.05$) in MHBW than LBW litters, except for brain weight, which was similar between LBW and MHBW litters Table 5-6). This resulted in significantly higher ($P<0.05$) brain:tissue weight ratios for LBW than MHBW litters (tendency for brain:adrenal weight ratio, $P=0.10$) (Table 5-6). Brain and spleen weight both showed an interaction between sex and litter birth weight category (Table 5-7) and brain:adrenal weight ratio showed an interaction between sex and treatment (Table 5-7). There was no interaction between treatment and litter birth weight category for any of the tissue weights or brain:tissue weight ratios.

There was an interaction between treatment and litter birth weight category for weaning weight and ADG ($P<0.05$). Both weaning weight (Figure 5-3a; $P<0.001$) and ADG (Figure 5-3b; $P<0.01$) were higher in MHBW litters than LBW litters from mLCPUFA-enriched sows, while there was no effect of birth weight category in litters from CON sows. Moreover, weaning weight tended to be higher ($P=0.08$) and ADG was significantly higher ($P<0.05$) in mLCPUFA than CON litters for MHBW litters, whereas there was no effect of treatment in LBW litters (Figure 5-3a and 5-3b). Pre-weaning mortality rate was similar between treatments (17.6% and 18.4% for CON and mLCPUFA, respectively, $P=0.83$), but was higher ($P<0.01$) in LBW (23.6%) than MHBW (15.7%) litters. There was no interaction between treatment and birth weight category for pre-weaning mortality rate.

5.3.3 Repeatability of litter average birth weight

Correlation analysis between litter average birth weight of the current litters and the three preceding litters within sows established a correlation ($P<0.001$) between litter average birth weight of the current litter and the previous litter ($r=0.49$), between the current litter and the previous 2 litters together ($r=0.49$), as well as between the current litter and the previous 3 litters together (0.50). The percent of sows in LBW, MBW and HBW categories in two consecutive farrowings is given in Figure 5-3 and indicates that none of the sows switched between the LBW and HBW categories in consecutive farrowings.

5.3.3 IgG concentration in serum and colostrum

Figure 5-5 shows the quadratic relationship between IgG concentration in serum and time to farrowing, which was significant for mLCPUFA ($P<0.05$) and was a trend for CON ($P=0.07$). There was no relationship between IgG concentration in colostrum and time from farrowing (data not shown). Therefore, the time to farrowing was included as a covariate in the analysis for serum, but not colostrum. There was no effect of treatment on IgG concentration in serum of sows around d113 of gestation (22.7 mg/ml vs. 21.8 mg/ml serum for CON and mLCPUFA sows, respectively; RSD=8.0, $P=0.68$), nor on IgG concentrations in colostrum within ~12 hours after farrowing (115.3 mg/ml vs. 137.3 mg/ml colostrum for CON and mLCPUFA sows, respectively: RSD=137.0, $P=0.43$) and there was no correlation between IgG concentration in serum and colostrum ($R=0.06$, $P=0.63$).

In litters with 9 to 16 total pigs born, IgG concentration in serum was again similar ($P=0.62$) at 113 days of gestation for mLCPUFA (22.1 mg/ml serum) and CON sows (23.1 mg/ml serum; RSD=7.2) and was also similar ($P=0.39$) for MHBW (21.4 mg/ml) and LBW sows (23.7 mg/ml; RSD=7.2). IgG concentration in colostrum of litters between 9 and 16 total pigs born was not different between treatments (122.6 mg/ml vs. 136.7 mg/ml colostrum for CON and mLCPUFA, respectively; RSD=149.2, $P=0.70$), or between litter birth weight categories (132.8 mg/ml vs. 126.5 ml/ml colostrum for LBW and MHBW sows, respectively: RSD=149.2, $P=0.88$).

5.4 Discussion

An increase in brain:liver weight ratio, indicative of brain sparing, and lower placental weight, have been suggested to be good measurements of intra-uterine growth retardation (Cooper, 1975; Town *et al.*, 2005) and the litters in the current trial classified as low birth weight (LBW) showed these benchmarks of intra-uterine growth retardation. However, marine-oil based n-3 LCPUFA enrichment did not result in differences in brain:liver weight ratio, or placental weight in litters between 9 and 16 total pigs born, suggesting that marine-oil based n-3 LCPUFA enrichment does not affect the processes related to IUGR. The fact that there was no interaction between treatment and birth weight category for placental weight or any of the tissue weights and brain:tissue weight ratios also supports this suggestion.

As described in Chapters 2 and 4, low litter average birth weight is believed to be a result of intrauterine crowding (IUC) in early gestation. During the embryonic implantation period, intrauterine competition occurs for the establishment of adequate surface area for nutrient exchange between the fetus and the mother (Foxcroft *et al*., 2006) and in IUC litters, an increased number of fetuses die after day 30 of gestation compared to normal litters. Indeed, Van der Lende and Schoenmaker (1990) showed that the importance of fetal mortality in pig populations increases with increasing ovulation rate, and as reviewed by Foxcroft *et al*. (2006), it has been suggested that commercial breeds with very high ovulation rates have high rates of early fetal loss, especially in multiparous sows. As ossification starts at the very early fetal stage, resulting in the presence of calcified bone tissue, dead fetuses are not resorbed by the sow but mummify instead. These mummified fetuses are expelled from the uterus at farrowing (as described by Van der Lende and Van Rens, 2003), but the smaller mummies can be easily missed. Nonetheless, we found a significant increase in number of mummies in LBW vs. MHBW litters in the current trial and, together with the tendency for higher number of stillborns in LBW than MHBW litters. This suggests a higher fetal death rate and problems with the farrowing process in low birth weight litters. The size of the mummies, which could indicate the time of death, was not measured in this trial.

Because of the importance of DHA for brain development, a higher brain weight for pigs born from marine-oil based n-3 LCPUFA enriched sows might have been expected. Indeed, Rooke *et al*. (2001c), who added salmon oil to sow diets in the amounts of 0, 5, 10 and 20 g/kg diet, showed a quadratic relationship between brain weight and salmon oil supplementation, so that brain weight increased when including salmon oil in the amounts of 0 to 10 g/kg, but decreased with the addition of 20 g/kg salmon oil. In the current trial, the diet was enriched with fish oil by 0.5% (5 g/kg), but brain weight measured in stillborn pigs from control and marine-oil based n-3 LCPUFA enriched sows was not different. However, the EPA and DHA content of Gromega was much higher than that of the salmon oil used by Rooke *et al*. (2001c), making it quite possible that the Gromega product would produce a response on the decreasing part of the quadratic relationship of brain weight to birth weight described by these authors, resulting in no difference in brain weight between piglets from control and marine-oil based n-3 LCPUFA enriched sows in the present study. None of the other piglet tissue weights

differed for piglets from marine-oil based n-3 LCPUFA enriched versus control sows, resulting in similar brain:tissue weight ratios in both treatment groups.

Litter average placental weight was not related to litter size. The fact that it was different in marine-oil based n-3 LCPUFA enriched sows compared to control sows in the entire dataset, but not in the subset of litters between 9 and 16 total pigs born, seemed to be due to a higher placental weight in a few small control litters, and not because of decreases in placental weight in small mLCPUFA litters. Furthermore, the low number of litters with less than 9 pigs born (4 mLCPUFA and 5 control) suggest caution in drawing conclusions about effects of marine-oil based n-3 LCPUFA supplementation on placental weight in small litters. On the other hand, the relationship between litter average birth weight and litter average placental weight in the entire dataset seems to be different between control and marine-oil based n-3 LCPUFA enriched sows, with mLCPUFA sows showing lower placental weights in low birth weight litters compared to control sows, and higher placental weights in high birth weight litters compared to control sows. This effect of marine-oil based n-3 LCPUFA supplementation on placental development and function merits further investigation.

The smaller litter size at birth that we observed, due to lower number of pigs born alive in litters from marine-oil based n-3 LCPUFA enriched sows versus control sows, was not consistent with our previous findings with the same supplement (Chapter 3) and reports from others. Most researchers found no effect of n-3 LCPUFA supplementation to sows on litter size at birth (Rooke *et al*., 2001a; Gunnarsson *et al*., 2009; Mateo *et al*., 2009; Leonard *et al*., 2010), while others found an increase in litter size (Webel *et al*., 2003; Spencer *et al*., 2004; Smits *et al*., 2011). Rooke et al. (2001c) showed a linear decrease in litter size with increasing amounts of salmon oil to the sows diets, but the authors concluded that this finding was unlikely to have been caused by the salmon oil inclusion, as they started supplementing sows at day 60 of gestation, well after the time period at which litter size in the pig is established.

It is not clear why the decrease in litter size in the marine-oil based n-3 LCPUFA enriched sows in the current trial occurred, but the eicosanoid PGE_2, of which AA is the precursor, might play a role. PGE_2 has been shown to affect progesterone levels and is important for other functions in early gestation. An increase in n-3 LCPUFA may have

been related with a decrease in n-6 LCPUFA availability, thus limiting PGE_2 production. PGE_2 stimulates progesterone secretion by cyclic CL, whereas PGE_1 (of which DGLA, also a n-6 PUFA, is the precursor) is the most potent stimulator of progesterone secretion in CL of pregnant ewes (Weems *et al*., 1997). It has been shown in cattle that n-3 PUFAs inhibit luteal cell progesterone secretion *in vitro* (Hinckley *et al*., 1996). Given that increases in n-3 LCPUFA usually result in decreased synthesis of n-6 LCPUFA, it could decrease PGE_1 and PGE_2 secretion, thus affecting progesterone secretion. Changes to the progesterone profile during the estrous cycle or during pregnancy could affect early embryo development, which in turn could decrease the number of embryos surviving to birth. Indeed, decreased progesterone concentration in the early luteal phase in cattle has been shown to reduce embryo survival (Mann *et al*., 1998). However, supplementing diets with fish meal (Mattos *et al*, 2002) or fish oil (Bilby *et al*., 2006) has not been shown to change luteal progesterone production in cows. Also in pigs, supplementing sow diets with fish oil, rich in EPA and DHA, did not affect circulating progesterone levels 60 to 72 hours after ovulation (see Chapter 3.3.3). It is, therefore, unlikely that the lower litter size at birth was related to changes in progesterone level during early pregnancy. PGE_2 has also been shown to play an important role in early gestation in promoting vascular permeability, placental development and the immune response of pigs (Kennedy, 1977; Geisert *et al*., 1990), and PGE_2 in the allantoic fluid has been related to larger litter size (Giguère *et al*., 2000). Brazle *et al*. (2009) showed that supplementing gilts from puberty onwards with the same fish oil product as used in the current trial increased DHA concentration in the chorioallantois, but the AA levels, which were expected to drop, were not different between supplemented and control gilts. This suggests again that the decrease in litter size is not likely due to changes in PGE_2 synthesis. More research is needed to understand the exact mechanisms by which n-3 LCPUFA affects the reproductive system, and why the outcome of n-3 LCPUFA supplementation to sows in terms of litter size are so variable.

When looking at the entire dataset, birth weight was similar between treatments, but body weight at weaning and ADG were higher in litters from marine-oil based n-3 LCPUFA enriched sows compared to control sows. This is in agreement with our previous findings (Chapter 3) and findings from other studies (Rooke *et al*., 2000 and 2001b; Mateo *et al*., 2009). For litters between 9 and 16 total pigs born, there was an interaction between treatment and litter birth weight phenotype, so that body weight (tendency) and ADG

were higher for MHBW but not for LBW litters from marine-oil based n-3 LCPUFA enriched sows. This outcome was inconsistent with our hypothesis that low birth weight litters would benefit more from marine-oil based n-3 LCPUFA supplementation than high birth weight litters. This hypothesis was based on the idea that high birth weight pigs already grow to their genetic potential, while low birth weight pigs have the potential to increase their postnatal growth performance. Based on the results reported in Chapter 3, nutritional supplementation with marine-oil based n-3 LCPUFA in gestation appeared to have the potential for low birth weight pigs to express more of their growth potential postnatally. Clearly, this was not the case. The question then becomes why marine-oil based n-3 LCPUFA supplementation did not produce the same postnatal growth benefits in LBW litters as it did in MHBW litters. In humans, it has been shown that placentae from IUGR (low birth weight) pregnancies decreased the flux of essential fatty acids and preformed LCPUFA to the fetus (Magnussen *et al*., 2004), and these placentae had decreased levels of AA and DHA, which lowered AA and DHA levels in the fetus relative to their LA and ALA precursors (Cetin *et al*., 2002). The decrease in flux of fatty acids in IUGR placentae was due to disrupted lipid metabolism and altered microvillous plasma membrane lipid hydrolase activities (Magnussen *et al*., 2004). Although the structure of the pig placenta is different from that of the human, it is possible that similar processes occur in the pig. If fatty acid transport to the fetus is decreased in LBW litters in the same manner as described for IUGR human placentae, this could be the reason why marine-oil based n-3 LCPUFA enrichment resulted in a lack of a positive response in body weight and ADG in LBW compared to MHBW litters. It could mean that, although EPA and DHA were higher in sow serum in all n-3 LCPUFA supplemented sows, they could not be transported to the fetus with the same efficiency in LBW as MHBW litters. It has been shown previously that feeding n-3 LCPUFA to gilts and sows during gestation increases DHA levels in the embryo (Chapter 3; Brazle *et al*., 2009). DHA is important for brain development (Innis, 2007) and in central dopamine metabolism (Ng and Innis, 2003), which in turn affects feeding behaviour (McEntee and Crook, 1991). A change in behaviour due to higher DHA levels in the brain could lead to increased postnatal growth rates. Indeed, Rooke *et al.* (2001b) showed that piglets of sows supplemented with n-3 LCPUFA from day 92 of gestation to term tended to contact the udder and grasped a teat more quickly than piglets from control sows, and that those piglets grew faster after birth, even though supplementation only occurred during gestation. This shows the importance of DHA availability in the fetus on postnatal growth performance. Therefore, it seems

reasonable to suggest that decreased efficiency in fatty acid transport in LBW litters compared to MHBW litters is one of the reasons why a positive response to marine-oil based n-3 LCPUFA supplementation in growth rates after birth was not seen. Post-weaning growth rates for low birth weight litters from sows fed with or without marine-oil based n-3 LCPUFA enrichment will be discussed in Chapter 6.

Although the absolute values of IgG concentration in sow serum reported in this trial were slightly higher than those reported by others (Devillers *et al*., 2004; Foisnet *et al*., 2010a), the decrease of IgG concentration in sow serum in the 2 days before farrowing was consistent with those reports (Devillers *et al*., 2004; Foisnet *et al*., 2010a), and the increase of IgG concentration after farrowing has also been found by Foisnet *et al*. (2010a). The large variation in serum IgG concentration between sows, as shown by the high residual standard deviation, was also observed by Foisnet *et al*. (2010a). Neither litter birth weight classification, nor marine-oil based n-3 LCPUFA enrichment of the sow had an effect on IgG concentration in sow serum before farrowing.

Compared to the serum samples, there was a much higher variation in IgG concentration in colostrum. This huge variation in colostral IgG concentration between sows may have been due to several factors. Firstly, most sows in this study were induced to farrow. It is not known if induction has an impact on colostral IgG concentration. However, considering that all IgG found in the colostrum comes from sow plasma/serum (Bourne and Curtis, 1973), that serum IgG concentration was already decreasing 2 days before farrowing, and that most sows farrowed within 24 hours of induction, it is possible that piglets are born when colostrum IgG concentration is not yet maximal, thus resulting in differences between induced and natural farrowing sows. Secondly, due to management challenges, up to 50% of the sows were moved to the farrowing rooms at day 114 of gestation and were given 15 mg altrenogest (Matrix, Intervet/Schering-Plough Animal Health, Millsboro, DE, USA) orally the morning of days 112 and 113 of gestation to prevent sows from farrowing in the gestation room. Foisnet *et al*. (2010b) showed that colostral IgG in sows receiving altrenogest from day 109 to 112 or 113 of gestation tended to decrease compared to sows not given altrenogest. Possibly, the altrenogest given to some sows at days 112 and 113 of gestation may have resulted in decreased colostral IgG concentrations. Thirdly, Devillers *et al*. (2004) have shown that colostrum IgG concentrations dropped quickly 10 hours after the start of farrowing. The samples in

the current trial were collected over a large time frame (-10 hours before to +25 hours after farrowing). There was no relationship between time from farrowing and IgG concentration in colostrum in this trial, but the time of farrowing was a rough estimation and might have been off by up to 6 hours during the night time farrowings. This does not help in an accurate identification of a relationship between time from farrowing and IgG concentrations, and thus, the time interval of sample collection may have played a role in the large variation in IgG concentration seen. Lastly, there were some challenges with the methodology of preparing the colostrum samples for the ELISA assay. Due to the small volumes of samples used, and the large dilution factors for both serum and colostrum, small mistakes in measuring volumes may have had a large impact. Moreover, due to the heterogeneous nature of the colostrum samples, variation in colostrum IgG concentration was especially high. Many duplicate samples had a CV higher than 20% in the first assays run. However, reruns showed that the thawing and refreezing process had degraded the protein in the samples. Only samples with a CV below 20% were used for analysis, but nonetheless, a large variation in IgG concentration between samples was seen, as shown by the high residual standard deviation. This made it difficult to detect any significant differences between treatments. Still, the numerical values may, with much caution, be compared with other research.

The lack of a correlation between IgG concentrations in serum and colostrum was also seen by Devillers *et al*. (2004). Although there was no statistically significant difference in IgG concentration between marine-oil based n-3 LCPUFA enriched and control sows in the present study, Mateo *et al*. (2009) found higher IgG concentrations in colostrum from n-3 LCPUFA supplemented sows, using the same fish oil product as in the current trial, and Rooke *et al*. (2003) reported enhanced piglet serum IgG concentration at weaning in piglets born to sows fed fish oil. The fact that Leonard *et al*. (2010) did not show higher IgG concentrations in colostrum from sows fed fish oil from 109 days of gestation onwards suggests that a longer period of supplementation before parturition is necessary to see beneficial effects of n-3 LCPUFA supplementation on IgG concentration in colostrum. Immunoglobulin concentration in colostrum is important due to the fact that maternal immunoglobulins transfer does not occur through the pig placenta (Le Dividich *et al*., 2005) and therefore, immunoglobulin intake by the piglet depends on sufficient colostrum intake. IgG is the main source of antibodies that boosts the neonatal pigs' passive immune system. Thus, increases of IgG concentration due to n-3 LCPUFA

supplementation may help survival and growth rates of the neonatal pig by improving their immune system.

In conclusion, this research showed that marine-oil based n-3 LCPUFA treatment decreased litter size at birth and weaning, and did not affect pre-weaning mortality rate. Over the entire population, marine-oil based n-3 LCPUFA supplementation increased ADG and body weight at weaning. However, there was an interaction between litter birth weight phenotype and marine-oil based n-3 LCPUFA treatment for litters between 9 and 16 total born, so that marine-oil based n-3 LCPUFA enrichment only improved growth rate in litters with medium or high birth weight, but not in litters with low birth weight. Although data on the IgG concentrations should be interpreted with caution, these results suggest that IgG concentration in serum at day 113 of gestation was not affected by n-3 LCPUFA enrichment of the sows' diet or litter birth weight classification.

Table 5-1. Ingredients and calculated nutritional composition of the standard gestation and lactation diets (% as-fed basis), with (mLCPUFA) or without (CON) enrichment with marine-oil based n-3 LCPUFAs

	Gestation		Lactation	
	CON	mLCPUFA	CON	mLCPUFA
Ingredients, %				
Corn	78.18	77.70	63.93	63.43
Soybean meal	17.10	17.10	31.35	31.35
Premixes	4.60	4.60	4.60	4.60
Vitamin E	0.13	0.13	0.13	0.13
Gromega Ultra 365 [a]	.	0.48	.	0.50
Calculated nutritional composition				
Digestible lysine, %	0.59	0.59	0.94	0.94
ME, MJ/kg	13.28	13.29	13.32	13.33
Ca, %	0.91	0.94	0.95	0.99
Available P, %	0.57	0.57	0.59	0.59
Vitamin A, KIU/kg	11.33	11.40	11.31	11.38
Vitamin D, KIU/kg	2.20	2.20	2.20	2.20
Vitamin E, IU/kg	128.04	133.67	127.23	133.14
Vitamin K, mg/kg	1.43	1.43	1.43	1.43
Crude fat, %	3.51	3.68	3.40	3.58
Total n-6 fatty acids, %	1.56	1.55	1.38	1.38
Total n-3 fatty acids, %	0.06	0.12	0.07	0.14

[a] Gromega Ultra 365 is a marine-oil based supplement rich in EPA and DHA. It is the same product as was used in Chapter 3, and its fatty acid profile is assumed to be close to that given in Table 3-2

Table 5-2. Characteristics at birth for litters born from sows being fed either diets with (mLCPUFA) or without (CON) marine-oil based n-3 LCPUFA enrichment

	CON	mLCPUFA	RSD	P-value
n	64	70		
Total born	13.4	11.9	3.4	0.01
Born alive	12.3	10.8	3.3	0.01
Born alive (% of total born)	92.0	90.8	12.2	0.60
Stillborn	1.1	1.1	1.5	0.95
Stillborn (% of total born)	8.0	9.2	12.2	0.60
Mummies	0.2	0.4	0.6	0.20
Litter ave bw (kg) [a]	1.44	1.40	0.16	0.25
Total litter bw (kg) [a]	17.72	17.24	1.88	0.19
Ave placental wt (kg) [b]	0.28 (n=37)	0.25 (n=37)	0.06	0.04

Data are the LSMeans, RSD=Residual standard deviation, ave = average, bw = birth weight

[a] Total number of pigs born in litter used as covariate

[b] Only taking into account litters where more than 50% of the placentae were recovered

Table 5-3. Tissue weights and brain:tissue weight ratios for necropsied pigs born to sows fed diets with (mLCPUFA) or without (CON) marine-oil based n-3 LCPUFA enrichment

	Treatment (Trt)		Sex		RSD	P-value	
	CON	mLCPUFA	Female	Male		Trt	Sex
n	66	65	59	72			
Birth weight (kg)	1.14	1.13	1.15	1.12	0.31	0.83	0.57
Tissue weights (g)							
Brain	27.41	27.30	27.38	27.33	2.23	0.86	0.90
Liver	37.56	36.00	36.99	36.58	12.74	0.53	0.86
Lung	25.13	25.05	26.81	23.37	8.55	0.96	<0.05
Heart	10.52	10.33	10.64	10.21	2.52	0.77	0.40
Small intestine	40.51	39.58	40.37	39.72	13.75	0.77	0.81
Kidney	9.42	9.88	10.07	9.23	2.78	0.45	0.12
Adrenal	0.27	0.26	0.27	0.26	0.07	0.78	0.36
Thymus	2.04	2.06	2.03	2.08	0.86	0.94	0.78
Spleen	1.30	1.31	1.33	1.28	0.43	0.83	0.57
	1.99	2.02	2.09	1.92	0.70	0.84	0.20
Semitendinosus muscle							
Brain:tissue weigh ratios							
Brain:liver	0.84	0.84	0.82	0.85	0.28	0.91	0.56
Brain:lung	1.25	1.24	1.14	1.35	0.41	0.86	<0.01
Brain:heart	2.86	2.86	2.79	2.93	0.68	0.99	0.28
Brain:intestine	0.80	0.81	0.76	0.85	0.30	0.83	0.13
Brain:kidney	3.30	3.02	2.98	3.34	0.97	0.16	0.05
Brain:adrenal	115.08	114.99	113.55	116.51	37.67	0.99	0.68
Brain:thymus	22.27	18.77	19.60	21.45	11.86	0.30	0.46
Brain:spleen	23.69	22.99	22.83	23.86	6.97	0.61	0.43
Brain:muscle	16.76	15.75	15.67	16.84	6.89	0.47	0.36

Data are the LSMeans, RSD=Residual standard deviation

There were no significant interactions between treatment and sex

Table 5-4. Litter information after cross-fostering for litters born to sows fed diets either with (mLCPUFA) or without (CON) marine-oil based n-3 LCPUFA enrichment

	CON	mLCPUFA	RSD	P-value
n	64	70		
Litter size at D1	11.4	10.3	1.0	<0.001
Litter size at D20	10.2	9.3	0.9	<0.001
Average litter weight D1 (kg) [a]	1.44	1.46	0.22	0.79
Total litter weight D1 (kg) [a]	18.26	18.37	2.44	0.81
Average litter weight D20 (kg) [b]	5.95	6.22	0.63	<0.05
Total litter weight D20 (kg) [b]	58.07	60.52	6.31	<0.05
Average daily gain (g) [b]	220	233	29	<0.05

Data are the LSMeans, RSD=Residual standard deviation

[a] Litter size at D1 was used as a covariate

[b] Litter size at D20 was used as a covariate

Table 5-5. Characteristics at birth for litters between 9 and 16 total pigs born with a low litter average birth weight (LBW) or medium/high litter average birth weight (MHBW) from sows being fed either diets with (mLCPUFA) or without (CON) marine-oil based n-3 LCPUFA enrichment

	Treatment (Trt)		BW phenotype (BW)			P-values	
	CON	mLCPUFA	LBW	MHBW	RSD	Trt	BW
n	48	49	25	72			
Total born (TB)	13.4	12.4	12.9	12.9	1.7	<0.01	0.96
Born alive	12.3	11.2	11.5	12.1	2.1	<0.05	0.28
Born alive (% of TB)	92.2	90.5	88.8	93.9	9.9	0.45	0.06
Stillborn	1.0	1.2	1.4	0.8	1.3	0.58	0.07
Stillborn (% of TB)	7.7	9.7	11.2	6.3	10.0	0.37	0.07
Mummies	0.4	0.5	0.7	0.2	0.7	0.78	<0.01
Litter ave bw (kg) [a]	1.32	1.27	1.11	1.50	0.11	0.20	<0.001
Total litter bw (kg) [a]	16.86	16.47	14.23	19.10	1.45	0.24	<0.001
Ave placental weight (kg) [b]	0.25 (n=26)	0.25 (n=24)	0.22 (n=9)	0.28 (n=41)	0.06	0.84	0.01

Data are the LSMeans, RSD=Residual standard deviation, ave = average, bw = birth weight

There were no significant interactions between treatment and birth weight category

[a] Total number of pigs born in litter used as covariate

[b] Only taking into account litters where more than 50% of the placentae were recovered

Table 5-6. Effect of marine-oil based n-3 LCPUFA supplementation to sows, sex and litter birth weight phenotype on tissue weights and brain:tissue weight ratios of necropsied pigs

	Treatment (Trt)		Sex		Litter birth weight phenotype (BW)			P-values		
	CON	mLCPUFA	Female	Male	LBW	MHBW	RSD	Trt	Sex	BW
n	44	44	38	50	31	57				
Birth weight (kg)	1.07	1.10	1.12	1.05	0.91	1.26	0.31	0.65	0.32	<0.001
Tissues (g)										
Brain [a]	27.30	27.38	27.70	26.99	27.03	27.65	2.00	0.91	0.22	0.41
Liver	36.14	34.60	36.57	34.17	30.48	40.26	11.57	0.60	0.37	<0.01
Lung	23.47	24.81	26.06	22.21	19.48	28.79	8.75	0.49	0.05	<0.001
Heart	10.00	10.10	10.29	9.81	8.48	11.63	2.75	0.88	0.44	<0.001
Small intestine	39.36	37.98	40.20	37.14	32.51	44.82	13.77	0.72	0.39	<0.01
Kidney	9.06	9.90	10.05	8.92	7.94	11.02	2.80	0.18	0.07	<0.001
Adrenal	0.27	0.26	0.27	0.26	0.24	0.29	0.08	0.71	0.36	<0.05
Thymus	1.91	1.95	1.96	1.90	1.38	2.48	1.02	0.90	0.80	<0.001
Spleen [a]	1.30	1.30	1.37	1.22	1.19	1.41	0.41	0.95	0.11	<0.05
Semitendinosus muscle	1.86	1.96	2.02	1.80	1.46	2.36	0.68	0.49	0.13	<0.001
Brain:tissue weight ratios										
Brain:liver	0.86	0.86	0.83	0.89	0.94	0.78	0.25	0.95	0.31	<0.05
Brain:lung	1.33	1.27	1.18	1.41	1.52	1.07	0.42	0.51	0.01	<0.001
Brain:heart	3.00	2.91	2.88	3.03	3.37	2.54	0.75	0.59	0.36	<0.001
Brain:intestine	0.83	0.85	0.78	0.91	0.98	0.70	0.30	0.83	0.09	0.001

	Treatment (Trt)		Sex		Litter birth weight phenotype (BW)			P-values		
	CON	mLCPUFA	Female	Male	LBW	MHBW	RSD	Trt	Sex	BW
Brain:kidney	3.36	3.01	2.96	3.42	3.67	2.70	0.90	0.08	<0.05	<0.001
Brain:adrenal [b]	115.76	113.98	113.14	116.60	122.87	106.87	36.72	0.85	0.69	0.10
Brain:thymus	25.30	20.71	21.70	24.30	30.40	15.61	14.49	0.25	0.47	<0.001
Brain:spleen	23.31	23.13	22.48	23.96	25.13	21.32	6.60	0.90	0.31	0.01
Brain:muscle	18.04	16.29	16.26	18.06	21.27	13.05	5.93	0.23	0.19	<0.001

Data are the LSMeans, RSD=Residual standard deviation

CON: sows fed control diets, mLCPUFA: sows fed diets rich in marine-oil based n-3 LCPUFA, LBW: low birth weight litters, MHBW: medium/high birth weight litters

[a] There was an interaction between sex and litter birth weight phenotype (P<0.05), see Table 5-7

[b] There was an interaction between treatment and sex (P=0.05), see Table 5-7

There were no three-way interactions between treatment, litter birth weight phenotype and sex

Table 5-7. Interaction between sex and litter birth weight phenotype, sex and treatment for tissue weights and brain:tissue weight ratios of necropsied pigs

	Female		Male	
	LBW	MHBW	LBW	MHBW
Brain weight (g)	28.16 [a]	27.23 [ab]	25.90 [b]	28.07 [a]
Spleen weight (g)	1.36 [a]	1.38 [a]	1.02 [b]	1.43 [a]
	CON	mLCPUFA	CON	mLCPUFA
Brain:adrenal weight ratio	122.54 [a]	103.74 [a]	108.98 [ab]	124.22 [b]

LBW: low birth weight litters, MHBW: medium/high birth weight litters, CON: sows fed control diets, mLCPUFA: sows fed diets rich in marine-oil based n-3 LCPUFA

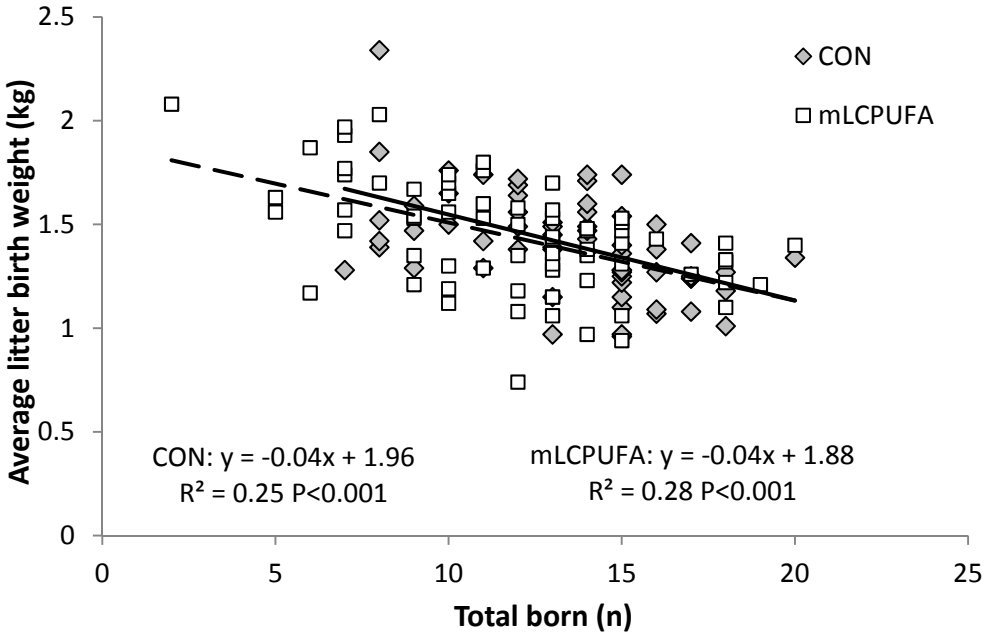


Figure 5-1. Relationship between litter size (total pigs born) and litter average birth weight for sows fed diets with (mLCPUFA, dotted line) or without (CON, solid line) marine-oil based n-3 LCPUFA enrichment (n=133)

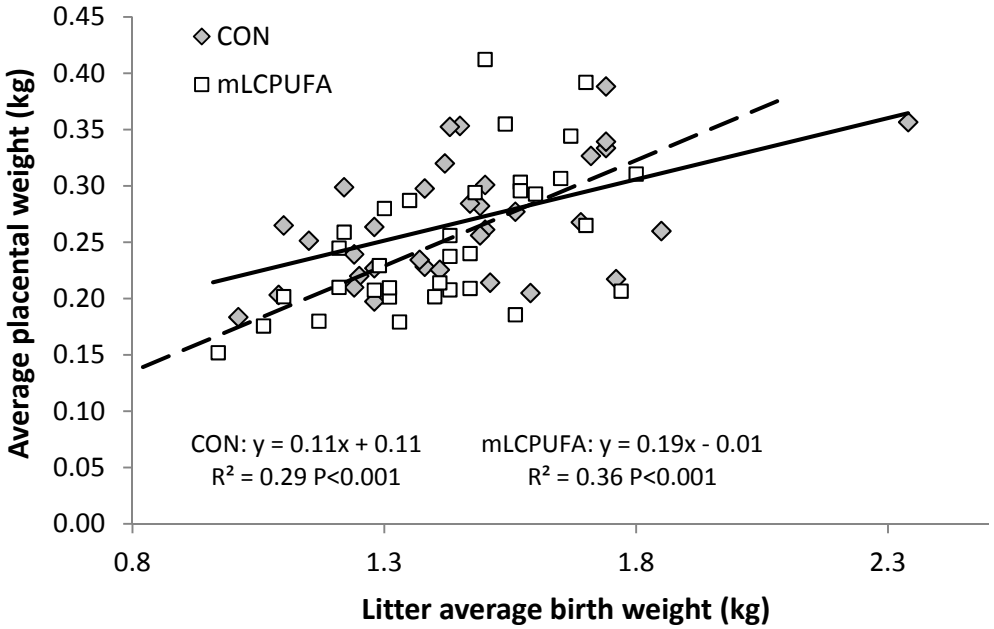
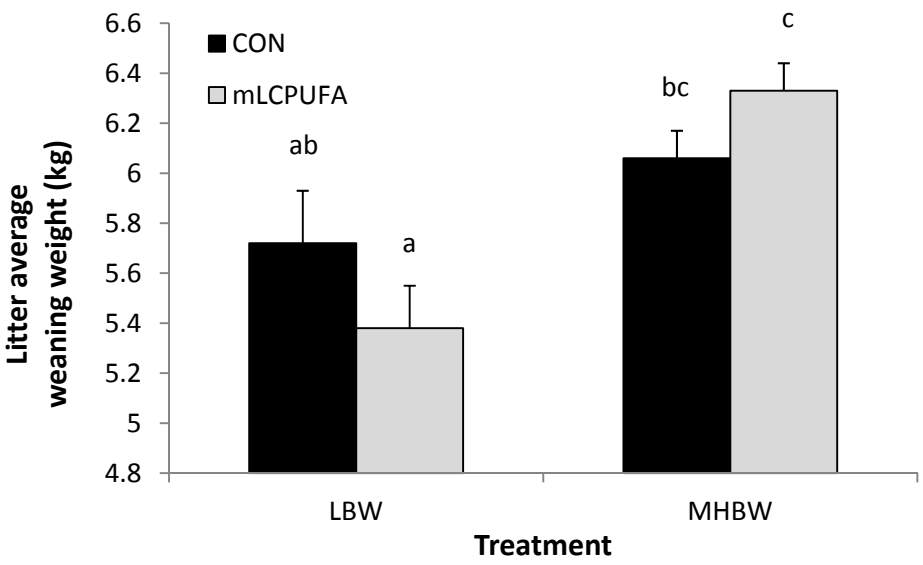
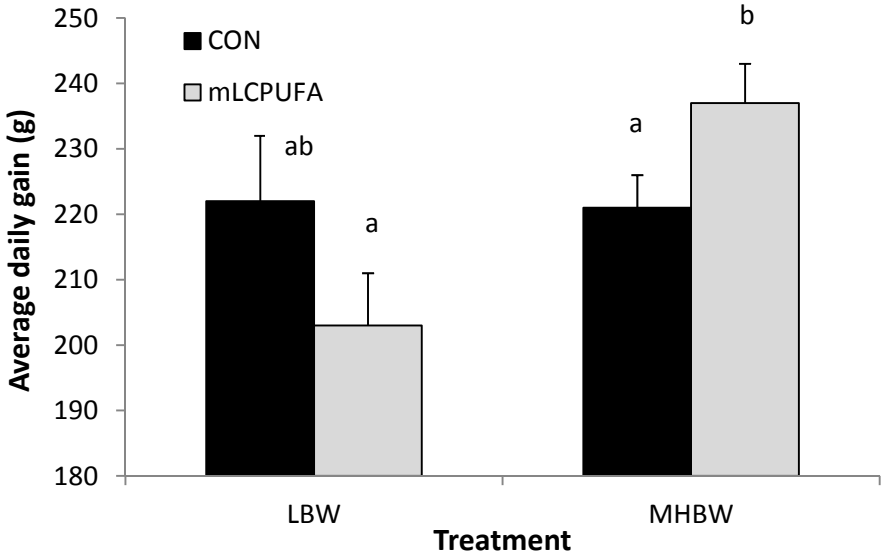


Figure 5-2. Relationship between litter average birth weight and litter average placental wet weight for litters from sows fed diets either with (mLCPUFA, dotted line) or without (CON, solid line) marine-oil based n-3 LCPUFA enrichment (n=74)



a.



b.

Figure 5-3. The interaction between litter average birth weight (LBW=low birth weight and MHBW= medium/high birth weight) and treatment (sows were fed diets either with (mLCPUFA) or without (CON) marine-oil based n-3 LCPUFA enrichment) for a) litter average weaning weight and b) average daily gain (n=97).

Columns without a common superscript are different at $P<0.05$.

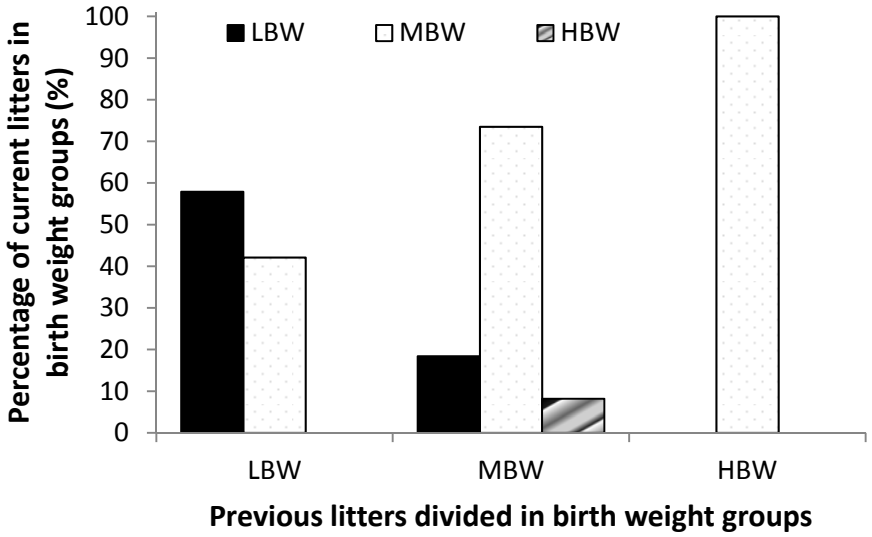


Figure 5-4. Classification of sows (n=78) into different birth weight categories (LBW = low birth weight, MBW = medium birth weight and HBW = high birth weight) in the last two recorded farrowings, showing the repeatability of litter average birth weight; none of the sows switch between the low and high birth weight category.

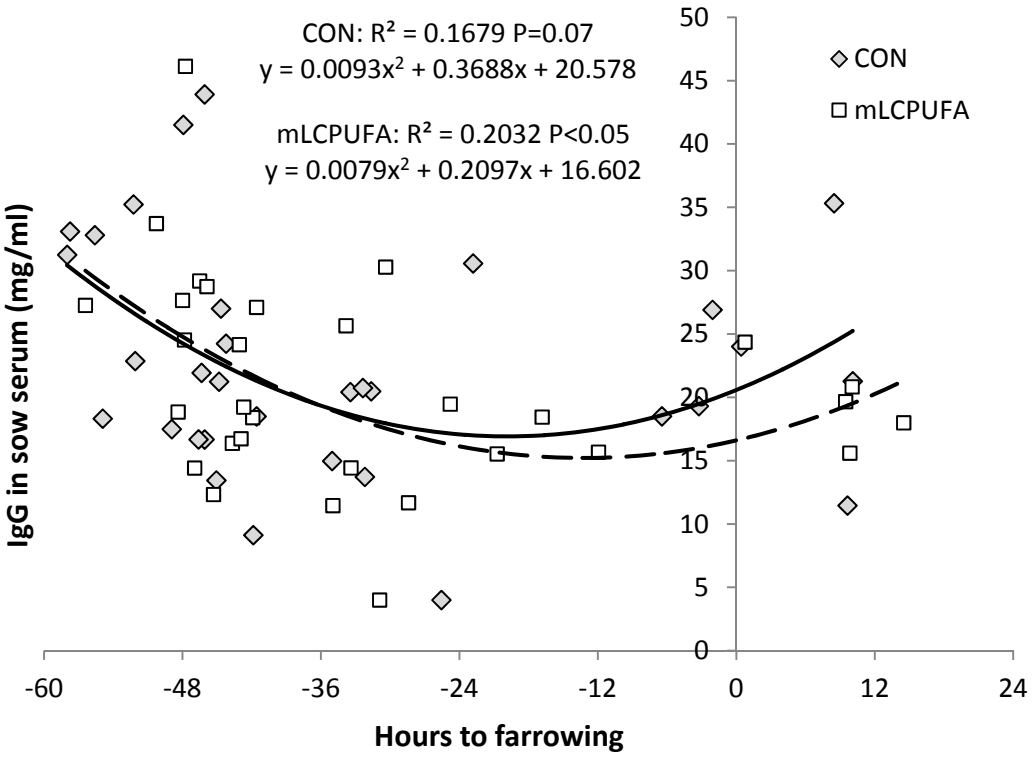


Figure 5-5. Relationship between IgG concentration in sow serum and time to farrowing for sows fed diets either with (mLCPUFA, dotted line) or without (CON, solid line) marine-oil based n-3 LCPUFA (n=64)

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Chapter 6: Dietary enrichment with a marine-oil based n-3 LCPUFA in sows with predicted birth weight phenotypes II: Effects on litter growth performance and carcass quality of offspring

6.1 Introduction

Growth uniformity after weaning is an important factor in the efficient use of all-in/all-out systems (Deen, 1997). Considering the lower growth rate in low birth weight litters found in Chapter 4, and the increased growth potential of pigs born from sows supplemented with marine-oil based n-3 LCPUFA found in Chapter 3, it was suggested that feeding marine-oil based n-3 LCPUFA to sows with a predicted low birth weight phenotype would increase the growth rate of their offspring. This would decrease the gap in growth rate between low and high birth weight litters and the variation in body weight at slaughter. However, before implementing a management strategy of only feeding marine-oil based n-3 LCPUFA to sows with a predicted low birth weight phenotype, it was important to investigate whether marine-oil based n-3 LCPUFA enrichment of the sow diet results in the same increase in growth rate in low birth weight litters as seen when feeding the entire sow population. Based on the results reported in Chapter 3, nutritional supplementation with marine-oil based n-3 LCPUFA in gestation appeared to have the potential for low birth weight pigs to express more of their growth potential post-natally.

The goal of this research was to study the effects of marine-oil based n-3 LCPUFA enrichment to the sow from weaning, during the rebreeding period, during gestation and until end of lactation, on growth performance and carcass quality of litters with a low birth weight phenotype. Maternal marine-oil based n-3 LCPUFA supplementation was hypothesized to increase growth performance of the low birth weight litters and improve carcass quality.

Chapters 5 and 6 describe results from the same trial. Chapter 5 described the effects of marine-oil based n-3 LCPUFA supplementation to all sows on trial on litter quality and growth until weaning, and explored the interactions between marine-oil based n-3

LCPUFA supplementation and litter birth weight phenotype. The present Chapter deals with subsets of lower average birth weight litters from sows fed control diets or diets supplemented with marine-oil based n-3 LCPUFA and describes data on their performance from birth, through the nursery and grow-finish periods, until slaughter, and assessed carcass quality. Fatty acid profiles of sow serum, milk and stillborn tissues were used to confirm adequate transfer of n-3 LCPUFA from the sows' diet to the offspring.

6.2 Material and methods

6.2.1. Animals and Treatments

The overall design of the experiment and the treatments applied were described in detail in Chapter 5. For the purposes of the analyses described in this Chapter, litters from four of the five breeding groups of sows receiving dietary treatment were selected to be studied from birth to weaning, in the nursery and grow-finish phases and at slaughter. Selected litters had between 9 and 16 total pigs born and the lowest litter average birth weight within each treatment (CON and mLCPUFA), and within each breeding group. Six litters per treatment per breeding group were selected, ranked by litter average birth weight, and then pair-matched between treatments based on this ranking. This provided a total of 24 litters with the lowest average birth weights per treatment for study. All results in this Chapter are based on these 48 selected litters. Results of litter performance from birth until weaning of all sows on trial were presented in Chapter 5.

6.2.2 Measurements before weaning

Within 24 hours after birth and before cross-fostering, sow ID, parity, date of birth, total number of piglets born, number of piglets born alive, number of stillborns, number of mummies, and individual birth weight and sex of all pigs born, were recorded for each litter.

Blood samples were taken by jugular venipuncture from all sows, one day before weaning, into non-heparinized vacutainer tubes (BD, Fisher Scientific, Ottawa, ON, Canada) and held at ambient temperature until centrifugation (Jorvet J-502) at 1034 x *g*. Serum was then harvested and frozen at -20°C. Colostrum samples were obtained from as many sows as possible within 12 hours, where possible, after the first piglet was born, without oxytocin administration. Colostrum samples were "milked" manually from all

teats, pooled within a sow, and then stored at -20°C until further analysis. Milk samples were also taken from as many sows as possible one day before weaning, when milk letdown was stimulated by intramuscular injection of oxytocin (VetTek Inc, Blue Springs, MO, USA) and the samples taken from two anterior and two posterior teats were pooled and stored at -20°C until further analysis.

6.2.3 Management after weaning

At weaning, the 48 selected litters were moved to nursery pens with 3 to 6 pigs per pen at the Burton Russell Farm (JBS United Inc.). All pigs in a litter were paired by individual birth weight and the pairs split between 2 pens, to establish 2 pens per litter with the same weight range. Nursery pens were 1.35 m^2.

At the end of the 5-week nursery period, 2 barrows and 2 gilts from each litter that had individual birth weights closest to their litter average birth weight, were moved to experimental grow-finish pens (barn A), where they were housed as 2 pigs per pen, sorted by sex within litter. Pens were 1.99 m^2. Remaining pigs in each litter were moved to another grow-finish barn (barn B) where they were kept in mixed-sex pens of up to 10 littermates. Pens were 11.15 m^2. After 8 weeks, one of the 2 pigs in each pen in barn A was relocated to the pens holding their respective litter mates in barn B. The barrow and gilt that initially had the individual birth weight closest to the litter average birth weight remained individually housed in the pens in barn A until slaughter.

A common, commercially available, four-phase nursery feeding program was utilized for the first 6 weeks after weaning for progeny originating from both sow treatment groups (Table 6-1) as follows; Pigs were fed a pelleted Phase 1 diet for the first week, followed by a succession of meal-based Phase 2, 3 and 4 diets, fed for one week, one week, and 3 weeks, respectively. Common grow-finish diets were corn and soybean meal based diets with added energy provided by the inclusion of 3% choice white grease (Table 6-2). Each phase was fed for 21 days until pigs were marketed.

6.2.4 Measurements after weaning

Body weight was measured on a pen basis at the start of the nursery period and after one and three weeks. Individual weights were then recorded at the end of the five-week nursery period. Average daily feed intake (**ADFI**), scour scores and mortality were also recorded on a pen basis. In the grow-finish phase, pigs in barn A were weighed

individually and pigs in barn B on a pen basis every 3 weeks, and on the day before slaughter. ADFI was measured in barn A only. Pigs in barn A were slaughtered at the same time as their littermates in barn B at an average litter live weight of 127 kg.

6.2.5 Fatty acid composition analysis in serum, milk and tissues

Of the 48 litters used in this study, 14 CON and 18 mLCPUFA litters had information of at least one necropsied pig available (see Chapter 5).Fatty acid composition of sow serum, whole colostrum, whole milk and tissues of brain, liver and *Semitendinosus* muscle from necropsied pigs (see Chapter 5) were analyzed for the litters that had at least one necropsied pig available. If more than one necropsied pig was available within a litter, the pig with the individual birth weight closest to the litter average birth weight was chosen for analysis. The tissue samples were kept frozen and ground with a mortar and pestle. Fatty acid analysis of all samples was performed as previously described for serum (Chapter 3.2). In short, a measured amount of sample (1 – 4 ml serum, 1 ml colostrum, 2 ml milk, 1.26 – 2.09 g brain, 1.95 – 3.57 g liver or 0.43 – 3.11 g *Semitendinosus* muscle) was used to extract lipids using methanol and chloroform in a 1:2 ratio. Lipids were evaporated to dryness under nitrogen. The extracted lipids were then transesterified using methanolic HCl (Sigma-Aldrich Inc., St. Louis, MO, USA). A 1:1.5 ratio of water and hexane was added, and after separation of the layers, the hexane layer was transferred to another tube containing a pinch of anhydrous sodium sulphate (Sigma-Aldrich Inc.). Samples were centrifuged and approximately 1 ml was transferred to chromatography vials.

Fatty acids were analyzed by a gas chromatograph (model Varian 3400; Varian Inc, Mississauga, ON), and used a flame ionization detector. It was equipped with a Varian 8100 auto sampler and using a SP-2560 fused silica capillary column (100 m x 0.25 mm i.d. x 0.2 µm film thickness; Supelco Inc., Bellefonte, PA). Hydrogen was the carrier gas. A cool on-column injection was used. The injector program started at 50 ºC and was immediately increased to 230 ºC at 150 ºC/min and held for 83 minutes. The column was operated at 45 ºC for 4 min, then temperature-programmed at 13 ºC/min to 175 ºC, held there for 27 min, programmed at 4 ºC/min to 215 ºC, and finally held there for 35 min; total run time was 86 min. The putative 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). Data was integrated using Galaxie Chromatography Data

System (Varian Inc., Mississauga, ON). 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. To calculate the actual amount of each fatty acid (μg/ml serum or μg/g tissue) an internal standard (C17:0) was used.

6.2.6 Statistical analysis

All variables were tested for normality prior to analyses, using the univariate procedure in SAS. Also the homogeneity of variance of the residuals was tested for each variable, using the Bartlett and Levine's tests in SAS. When variables were not normally distributed, they were only transformed if the variance of residuals was not homogeneous and if it improved the fit of the model. None of the variables needed transformation on this basis.

All data collected before weaning were analyzed using the MIXED procedure of SAS (SAS Institute, Cary, NC, USA) as a randomized incomplete block design, with blocks based on sow pairs. The model included sow treatment (CON or mLCPUFA) as a fixed effect and pair as a random effect. Sow was used as the experimental unit for all parameters before weaning, including treatment effects on litter growth, and all individual measurements of piglets before weaning were averaged within a litter (sow) before statistical analysis. For nursery data, a nested design was used for pig growth and feed intake, with pen-within-sow as the experimental unit. The model included sow treatment and sex as a fixed effect. Litter pairing at weaning was used as a random effect. Repeated measures analysis was used for piglet body weight. An appropriate covariance structure was selected by comparing the goodness-of-fit measures of different structures. The Kenwardroger approximation was used for the denominator degrees of freedom.

In the grow-finish phase, data of barn A were again set up as a nested design, with pen-within-sow as the experimental unit, while barn B was set up as a randomized incomplete block design, with blocks based on litter pairing at weaning. Individual carcass data of both barns was analyzed as a nested design, with pig-within-litter as the experimental unit. Categorical data like scour scores and pre-weaning mortality were analyzed separately, using the generalized logit function (proc CATMOD in SAS). Data in the text and Figures are given as least square means, unless otherwise stated. Probability values < 0.05 were considered significant and values < 0.10 were used to describe trends.

6.3 Results

6.3.1 Birth until weaning data

The total number of pigs born and born alive were lower (P=0.05) in mLCPUFA than CON sows, while the number of stillborn and mummified pigs were similar between treatments (Table 6-3). Number of stillborns and pigs born alive as a percentage of total litter size was not different between treatments (Table 6-3). Average placental weight was similar in mLCPUFA and CON sows (Table 6-3). Pre-weaning mortality rate was not different between treatments (23.5% and 18.4% for CON and mLCPUFA respectively, P=0.13).

6.3.2 Nursery data

The number of pigs per pen, ADG, ADFI and feed efficiency (pen feed intake/pen weight gain) were similar between treatments (Table 6-4). Overall body weight over time in the nursery was not different between treatments, and no interaction between treatment and time on body weight occurred (Figure 6-1). Mortality rate and morbidity rate (calculated as the number of pigs receiving individual medication), and scour scores in the nursery (1 = no scours, 2 = mild scours, 3 = severe scours: average score for progeny from CON = 1.07 and from mLCPUFA-enriched sows = 1.08, respectively; P=0.65) were also not different between treatments (Table 6-4).

6.3.3 Grow-Finish and carcass data barn A

There was a significant interaction between treatment and time for body weight (P<0.01). Body weight was not different at the start of, and in the first half of the grow-finish phase, but was higher (P < 0.01) for mLCPUFA than CON pigs in the second part of the grow-finish phase (Figure 6-2). However, ADG, ADFI and feed efficiency were similar between treatments in barn A (Table 6-5 and Figure 6-3) and mortality rate in barn A was not different between treatments (0% for CON and 2.1% for mLCPUFA, P=0.60).
By design, slaughter weight was similar between treatments (Table 6-5). However, both the actual age at slaughter, and the calculated age at the fixed slaughter weight of 127 kg, were also not different between treatments (Table 6-5). Of the 47 CON pigs, two were not shipped to the slaughterhouse and one was rejected in the slaughterhouse. Of the 48 mLCPUFA pigs, one was not shipped to the slaughterhouse, and the percentage of pigs

slaughtered was similar between treatments (P=0.32). Carcass weights were similar between treatments (Table 6-6) and there was also no difference in loin depth, lean meat percentage, yield percentage, premium paid and sort loss (money lost on the carcass due to lower quality) between treatments (Table 6-6). There was an interaction (P<0.01) between treatment and sex for fat depth. Fat depth was higher (P=0.01) in females from mLCPUFA-enriched sows (18.55 ± 0.88 mm) than from CON sows (15.31 ± 0.94 mm), whereas there was no difference in fat depth between treatments in males (19.71 ± 0.90 mm and 21.15 ± 0.90 mm for males from mLCPUFA-enriched and CON sows, respectively: P=0.25).

6.3.4 Grow-Finish and carcass data barn B

Number of pigs per pen was not different between treatments (Table 6-7). Start and end weight, ADG, age at slaughter and calculated age at a fixed slaughter weight of 127 kg were also not different between treatments (Table 6-7 and Figure 6-3). Overall, body weight was not different between treatments, and there was no interaction between body weight and time (Figure 6-4). Mortality rate in barn B was similar between treatments (1.1 % and 1.2 % for progeny from CON and mLCPUFA-enriched sows, respectively, P=0.95).

Of the 180 pigs from CON sows, 6 were not slaughtered (3.3 %), whereas 14 of the 169 pigs from mLCPUFA-enriched sows were not slaughtered (8.3 %), with the percentage of pigs slaughtered being lower in litters from mLCPUFA-enriched than CON sows (P=0.05). Live weight and carcass weight were similar between treatments (Table 6-8). Fat depth was higher (P=0.01) and loin depth tended to be lower (P=0.08) in pigs from mLCPUFA-enriched sows compared to pigs from CON sows, which resulted in a lower lean meat percentage (P<0.01) for pigs from mLCPUFA-enriched sows (Table 6-8). There was no difference in yield percentage, premium paid and sort loss between treatments (Table 6-8).

6.3.5 Fatty acid concentration in serum, colostrum, milk and stillborn tissues

mLCPUFA sows had increased (P<0.01) EPA and DHA concentrations in serum, colostrum and milk compared to CON sows (Table 6-9 and 6-10). This resulted in an increased total concentration of n-3 LCPUFA, and a decreased n-6:n-3 ratio in mLCPUFA vs. CON sows (Table 6-9 and 6-10).

DHA concentration was also increased (P<0.05) in brain, liver and *Semitendinosus* muscle from stillborn piglets born to mLCPUFA sows compared to CON sows (Table 6-11 and 6-12). EPA concentration was higher (P<0.05) for stillborns born to mLCPUFA than CON sows in liver and muscle (as % of total fatty acids only), but was not affected in the brain. Again, total n-3 LCPUFA concentration was increased and n-6:n-3 ratio decreased in the measured tissues from stillborns born to mLCPUFA vs. CON sows (Table 6-11 and 6-12).

6.4 Discussion

Although brain weight was not different between treatments (see Chapter 5), the DHA composition of brain tissue was higher in stillborns from marine-oil based n-3 LCPUFA fed sows compared to controls, which is in agreement with other research (Rooke *et al.,* 2000, 2001b). DHA concentration was also increased in liver and *Semitendinosus* muscle tissues of pigs from treated sows, which is again in agreement with other reported findings (Rooke *et al*., 2000, 2001b; Missotten *et al.,* 2009). This shows that DHA must have been available to the fetus during gestation. This is in agreement with our previous findings that EPA and DHA were higher in sow serum during gestation, and that DHA concentration was increased in embryos at day 30 of gestation (Chapter 3). Effects of maternal marine-oil based n-3 LCPUFA supplementation on EPA concentration were variable between tissues. EPA was increased in liver, which is in agreement with Rooke *et al.* (2000 and 2001b) and Missotten *et al.* (2009). EPA was increased in the *Semitendinosus* muscle, but only when expressed as percent of total fatty acids, while Missotten *et al.* (2009) found increased EPA when expressed as mg/100g tissue. EPA concentration was not affected by maternal marine-oil based n-3 LCPUFA supplementation in the brain, which is not in agreement with previous findings (Rooke *et al.* 1999, 2000 and 2001b). Increases in EPA and DHA concentration in sow serum during lactation are consistent with results from Fritsche *et al.* (1993). EPA and DHA concentration were also increased in colostrum and milk, again consistent with previous findings (Taugbol *et al.,* 1993; Fritsche *et al.,* 1993; Rooke *et al.,* 2000; Leonard *et al.,* 2010).

Consistent with the findings reported in Chapter 4, pre-weaning mortality was very high in the subsets of lower birth weight litters studied, regardless of treatment. Although the

effect of marine-oil based n-3 LCPUFA enrichment of the sow was not significant, it did decrease pre-weaning mortality by 5 percent points, which translates into at least half a pig more at weaning in litters of 10 or more pigs. If this trend was substantiated in larger studies it could be of economic importance in practice. However, there is presently no clear consistency in the literature on the effects of n-3 LCPUFA supplementation on pre-weaning mortality. Rooke *et al.,* (2001a) showed that inclusion of salmon oil (rich in n-3 LCPUFA) in the sow's diet decreased pre-weaning mortality from 11.7% to 10.2%, mainly due to a reduction in piglets crushed by the sow. However, in both the study described in Chapter 3 and in the study of Rooke *et al*. (2000), pre-weaning mortality was higher in marine-oil based n-3 LCPUFA supplemented sows than control sows. As discussed in Chapter 3, interpretation of effects of marine-oil based n-3 LCPUFA treatment on survivability in this experiment may be difficult due to confounding effects of gestation length and the use of induced farrowing, as was again the practice in the present trial.

Gabler *et al*. (2009) showed that ex-vivo glucose uptake in the jejunum of pigs at weaning was substantially increased when the sows were fed with n-3 LCPUFA during gestation and/or lactation. The n-3 LCPUFA supplementation in gestation seemed more important in this effect than n-3 LCPUFA supplementation during lactation, as n-3 LCPUFA supplementation in gestation alone improved glucose uptake by about 400%, while n-3 LCPUFA supplementation in lactation alone did not significantly increase the glucose uptake. However, the best result was seen when feeding n-3 LCPUFA during both gestation and lactation, which improved glucose uptake by 500%. An increase in glucose uptake could result in higher growth rates, and this is one of the mechanisms discussed in Chapter 3 by which n-3 LCPUFA supplementation to sows could affect offspring growth rates. As shown in Chapter 5, in the overall data set there was an interaction between marine-oil based n-3 LCPUFA treatment and litter birth weight classification, so that marine-oil based n-3 LCPUFA enrichment of the sow improved ADG and body weight at weaning in pigs from medium or high birth weight, but not low birth weight litters. As most of the litters allocated to the current detailed study of post-weaning growth performance were from the lower rankings of birth weight, the lack of a response to marine-oil based n-3 LCPUFA enrichment of these sows on ADG and body weight at weaning was not surprising. As discussed in Chapter 5, it is possible that fatty acid transfer in low birth weight pigs is not as efficient as in medium or high birth weight

pigs, and therefore, less of the DHA would reach those fetuses. This would mean that glucose uptake is not improved to the same extent for low birth weight litters compared to medium or high birth weight litters. Nonetheless, the increased body weight found in litters from marine-oil based n-3 LCPUFA enriched sows in barn A in the later periods of the grow-finish phase might still relate back to improvements in gut development at weaning.

As most pigs included in the present trial had low birth weights, the data presented in Chapter 5 would suggest that this is due to IUGR. D'Inca *et al*. (2011) showed that IUGR pigs had a reduction in the surface area of exchange in the gut of more than 60% during the first days of life, as estimated by the combined reduction of small intestinal length, mucosa density, dry matter content and villous sizes. Moreover, Alvarenga *et al*. (2013) showed that low birth weight pigs within a litter had a lower height of the duodenal mucosa layer at birth compared to pigs with a high birth weight, and that this difference persisted until 150 days of age. These studies clearly show the negative effects of low birth weight on intestinal morphology, which could contribute to growth failure, a well-known morbidity associated with IUGR (D'Inca *et al*., 2011). On the other hand, Leonard *et al*. (2011) have shown that feeding n-3 LCPUFA from day 109 of gestation until weaning increased villous height and villous height to crypt depth ratio in the jejunum and ileum mucosal surface, which is an important indicator of gut health, and these improvements were seen at 9 days after weaning. Thus, the uptake of n-3 LCPUFA through milk had lasting effects on gut health. A decrease in scour scores in the nursery phase might have been expected if marine-oil based n-3 LCPUFA supplementation of sows improved gut development of piglets, but scour scores were not different between treatments in the current trial, in which most scours occurred in the first week after weaning. Possibly, the weaning process was just too stressful for marine-oil based n-3 LCPUFA to have an effect.

The results for body weight and ADG in the grow-finish phase were different between barn A and B. Although the ADG was not statistically compared between barns, Figure 6-3 shows that barn B had higher ADG in the 2nd and 3rd period of the grow-finish phase than barn A. During these periods, there were 2 pigs / pen in barn A. At the end of the 3rd period, one of the 2 pigs of each pen were moved from barn A and reunited with their littermates in barn B, after which time ADG did not increase in barn B, but did increase

in barn A for remaining pigs born to sows fed marine-oil based n-3 LCPUFAs. This suggests that marine-oil based n-3 LCPUFA supplementation of sows with a lower birth weight phenotype only improves growth performance of their offspring if pigs are not dealing with any competition for food or space in a pen. And even though body weight was higher later in the grow-finish phase in barn A for pigs born from marine-oil based n-3 LCPUFA fed sows vs. control sows, and led to a decrease of 5 days in the calculated age at a fixed slaughter weight of 127 kg, this calculated age was not significantly different between treatments. In barn B no effect of treatment was seen on age at market or calculated age at a fixed market weight of 127 kg. Together with the observation that marine-oil based n-3 LCPUFA enrichment did not affect ADG, ADFI or feed utilization efficiency, this suggests that in a commercial setting, supplementing sows with a predicted low birth weight phenotype with marine-oil based n-3 LCPUFA during gestation and lactation would not be a good management strategy.

To our knowledge, nobody to-date has looked at the indirect effect of maternal marine-oil based n-3 LCPUFA supplementation on carcass traits of the offspring. Sousa *et al*. (2010) directly fed pigs from 68 kg until 100 kg body weight with a diet with different added fat sources; soybean oil, canola oil, linseed oil or a commercial PUFA oil. Treatments did not have an effect on ADG, ADFI, feed utilization efficiency, average fat thickness, carcass length, carcass yield percentage, loin area muscle or meat to fat ratio. Subsequently, Wojtasik1 *et al*., (2012) fed pigs from 60 to 105 kg body weight with one of three diets: Diet A contained 1% rapeseed oil, 2% fish oil, and 0.5% lard; diet B contained 2.5% rapeseed oil and 1% linseed oil; and diet C contained 2.5% linseed oil and 1% fish oil. There were no differences between fat mixtures in ADG, whole carcass weight, primal cuts, subcutaneous adipose tissue, or meat and total fat content in the carcass. This would suggest that maternal n-3 LCPUFA enrichment would also not result in changes in carcass traits. However, in one study that looked at effects of different levels and sources of lipids (but not n-3 LCPUFA) to sows during pregnancy and lactation on meat quality of the offspring, Gerfault *et al*. (2000) reported that inclusion of 2.9% copra oil, sunflower oil or lard during the whole of pregnancy and lactation did not affect growth performance and carcass quality of their progeny at 100 kg. However, the number of adipocytes per gram of adipose tissue of the pigs at 100 kg was increased when the maternal diet had been supplemented with lipids. They concluded that the quantity of fat added to sow diets could affect fat content in the carcass of their offspring.

Inclusion of n-3 LCPUFA in the sow's diet might, therefore, also increase fat content in the carcass of the offspring. Indeed, fat depth was higher in pigs from marine-oil based n-3 LCPUFA-enriched sows than from control sows in barn B, and there was a similar, but sex-specific increase in fat depth in females from marine-oil based n-3 LCPUFA enriched sows in barn A. Other differences between barn A and B included a tendency for decreased loin depth and significantly decreased lean meat percentage in pigs of marine-oil based n-3 LCPUFA enriched sows compared to controls in barn B, while these variables were not affected by maternal marine-oil based n-3 LCPUFA enrichment in barn A.

Chapter 4 discussed different research findings on the effect of piglet birth weight on carcass quality. Some researchers found higher backfat thickness or higher adipose tissue yield in low birth weight pigs compared to high birth weight pigs (Gondret *et al*. 2006; Bee, 2004), while others did not show differences in backfat or adipose tissue yield when pigs were slaughtered at a similar weight (Wolter *et al*., 2002; Gondret *et al*., 2005; Chapter 4). Results for lean meat percentage were also inconsistent; Gondret *et al*. (2006) found lower lean meat percentage in low than high birth weight pigs, while Wolter *et al*. (2002), Bee (2004) and Gondret *et al*. (2005) did not show any differences in lean meat percentage between birth weight categories. The current trial hoped to establish a positive effect of maternal marine-oil based n-3 LCPUFA enrichment on offspring carcass quality, to offset the possible negative effects of low birth weight on carcass quality. Instead, maternal marine-oil based n-3 LCPUFA enrichment increased fat depth, tended to decrease loin depth and decreased lean meat percentage. In other words, the marine-oil based n-3 LCPUFA enrichment of sows resulted in a negative effect on exactly the same parameters that were also negatively affected by low birth weight. Therefore, it can be concluded that marine-oil based n-3 LCPUFA enrichment of sows is not a good mechanism to improve carcass quality in low birth weight litters.

To summarize, marine-oil based n-3 LCPUFA enrichment of sows from weaning, during rebreeding, during gestation and until the end of lactation, increased EPA and DHA concentration in sow serum, colostrum and milk. It also increased DHA concentration in the brain, liver and *Semitendinosus* muscle, and EPA concentration in liver and muscle, of stillborn pigs. However, supplementation of the sow until the end of lactation did not affect ADG, ADFI or feed utilization efficiency of the offspring in the lower birth weight

litters studied. Body weight was higher in the second half of the grow-finish phase in pigs from marine-oil based n-3 LCPUFA fed sows compared to controls, but only when space and competition for feed was minimal, as in barn A, and maternal marine-oil based n-3 LCPUFA enrichment had some effects on carcass fat depth, loin depth and decreased lean meat percentage. In conclusion, these results collectively suggest that nutritional supplementation of the sow can have lasting effects on litter development, but that feeding marine-oil based n-3 LCPUFA to sows during gestation and lactation was not effective in improving growth rates or carcass quality of low birth weight litters.

Table 6-1. Calculated chemical composition of the nursery diets fed to all pigs on trial

	Phase 1	Phase 2	Phase 3	Phase 4
Days fed	7	7	7	21
Digestible lysine (%)	1.45	1.40	1.38	1.28
ME (MJ/kg)	14.99	14.03	14.23	14.77
Vitamin E (IU/kg)	110.23	92.59	92.59	68.34

Table 6-2. Ingredients and calculated nutritional composition (% as-fed basis) of the grow-finish diets fed to all pigs on trial

	Phase 1	Phase 2	Phase 3	Phase 4	Phase 5
Number of days fed	21	21	21	21	To slaughter
Ingredients, %					
Corn	62.6	69.1	74.6	78.2	81.1
Soybean meal	24.4	18.7	13.6	10.8	7.9
Fat – Animal lard	3	3	3	3	3
Base mix	10	9.2	8.8	8	8
Calculated nutritional composition					
Digestible lysine, %	1.23	1.05	0.91	0.80	0.73
ME, MJ/kg	14.30	14.30	14.30	14.31	14.30
Ca, %	0.61	0.55	0.51	0.46	0.46
Available P, %	0.29	0.26	0.25	0.23	0.22
Vitamin A, KIU/kg	4.34	4.01	3.86	3.53	3.53
Vitamin D, KIU/kg	0.99	0.90	0.88	0.79	0.79
Vitamin E, IU/kg	83.00	77.21	74.43	68.48	68.63
Vitamin K, mg/kg	0.53	0.49	0.46	0.42	0.42
Crude fat, %	6.34	6.39	6.43	6.46	6.48
Total n-6 fatty acids, %	1.67	1.75	1.82	1.86	1.9
Total n-3 fatty acids, %	0.10	0.10	0.09	0.09	0.08

Table 6-3. Birth to weaning data for litters with a low average birth weight from sows fed either control diets (CON) or diets enriched with marine-oil based n-3 LCPUFAs (mLCPUFA)

	CON	mLCPUFA	RSD	P-value
n	24	24		
Total born (TB)	13.7	12.7	1.3	0.05
Born alive	13.1	11.8	2.1	0.05
Born alive (% of TB)	96.2	93.2	8.7	0.26
Stillborn	0.5	0.8	1.1	0.43
Stillborn (% of TB)	3.8	6.8	8.7	0.26
Mummies	0.3	0.5	0.7	0.24
Litter ave bw (kg)	1.31	1.29	0.12	0.68
Total litter bw (kg) [a]	17.32	16.71	1.83	0.33
Ave placental weight (kg) [b]	0.26 (n=12)	0.25 (n=14)	0.05	0.62
Weaning weight (kg)	5.65	5.77	0.46	0.44
Average daily gain (g)	207	215	18	0.24
Number pigs weaned	10.2	9.6	1.6	0.28

Data are the LSMeans, RSD=Residual standard deviation, ave = average, bw = birth weight

There were no significant interactions between treatment and birth weight category

[a] Total number of pigs born in litter used as covariate

[b] Only taking into account litters where more than 50% of the placentae were recovered

Table 6-4. Nursery data for litters with a low average birth weight from sows fed either control diets (CON) or diets enriched with marine-oil based n-3 LCPUFAs (mLCPUFA).

	CON	mLCPUFA	RSD	P-value
n	45	46		
Number of pigs per pen	5.2	4.9	0.4	0.26
ADG (kg)	0.34	0.37	0.03	0.13
ADFI (kg)	0.45	0.47	0.05	0.27
Feed efficiency (feed/gain)	1.30	1.29	0.04	0.11
Mortality (%)	1.29	0.89	.	0.69
Morbidity (%) [a]	14.35	11.26	.	0.33

Data are the LSMeans, RSD=Residual standard deviation

[a] Calculated as number of pigs given individual medication

Table 6-5. Grow-Finish data of barn A for litters with a low average birth weight from sows fed either control diets (CON) or diets enriched with marine-oil based n-3 LCPUFAs (mLCPUFA) [a]

	Treatment (Trt)		Sex			P-value	
	CON	mLCPUFA	Female	Male	RSD	Trt	Sex
n	47	48	48	47			
ADG (kg)	0.79	0.81	0.77	0.83	0.08	0.16	<0.001
ADFI (kg)	2.07	2.10	1.99	2.18	0.16	0.51	<0.001
Feed efficiency (feed/gain)	2.63	2.59	2.58	2.64	0.18	0.44	0.19
End weight (kg)	126.0	128.6	123.0	131.6	12.0	0.31	<0.001
Age at market (d)	173.3	172.3	172.9	172.8	0.4	0.60	0.57
Calculated age at market at 127 kg (d)	176.6	171.2	178.8	168.9	17.4	0.12	<0.01

Data are the LSMeans, RSD=Residual standard deviation

[a] Data is based on pens with 2 pigs/pen in the first 8 weeks, then 1 pig/pen until slaughter

No significant interactions between treatment and sex occurred

Table 6-6. Carcass data for littermates housed in barn A selected from low average birth weight litters born to sows fed either control diets (CON) or diets enriched with marine-oil based n-3 LCPUFAs (mLCPUFA),

	Treatment (Trt)		Sex			P-value	
	CON	mLCPUFA	Female	Male	RSD	Trt	Sex
n	44	47	45	46			
Live weight (kg)	126.9	129.4	124.0	132.4	9.1	0.24	<0.001
Hot carcass weight (kg)	94.6	96.3	92.7	98.3	7.8	0.33	0.001
Fat depth (mm) [a]	18.23	19.13	16.93	20.43	4.16	0.30	<0.001
Loin depth (mm)	69.86	69.84	70.65	69.05	6.05	0.99	0.22
Lean meat (%)	55.4	55.2	55.8	54.7	1.6	0.55	<0.01
Yield (%)	74.5	74.4	74.8	74.1	3.2	0.84	0.35
Grade premium (US$)	6.23	6.39	6.57	6.05	1.62	0.71	0.13
Sort loss (US$)	-1.06	-1.37	-0.60	-1.83	1.64	0.37	<0.001

Data are the LSMeans, RSD=Residual standard deviation

[a] There was a significant interaction for treatment and sex for fat depth (P<0.01), see text. No other significant interactions between treatment and sex occurred

Table 6-7. Grow-finish data for littermates housed in barn B from low average birth weight litters born to sows fed either control diets (CON) or diets enriched with marine-oil based n-3 LCPUFAs (mLCPUFA)

	CON	mLCPUFA	RSD	P-value
n	24	24		
Number of pigs per pen	5.8	5.3	1.7	0.41
Start weight (kg)	17.8	18.3	2.3	0.48
ADG (g)	0.96	0.96	0.06	0.74
End weight (kg)	128.9	128.0	5.8	0.61
Age at market (d)	173.3	172.4	6.5	0.61
Calculated age at market at 127 kg (d)	172.4	171.8	6.8	0.75

Data are the LSMeans, RSD=Residual standard deviation

No significant interactions between treatment and sex occurred

Table 6-8. Carcass data for littermates housed in barn B from low average birth weight litters born to sows fed either control diets (CON) or diets enriched with marine-oil based n-3 LCPUFAs (mLCPUFA)

	Treatment (Trt)		Sex (S)			P-value	
	CON	mLCPUFA	Female	Male	RSD	Trt	S
n	174	155	165	164			
Live weight (kg)	123.0	124.4	119.3	128.2	11.9	0.62	<0.001
Hot carcass weight (kg)	94.3	95.1	91.2	98.2	9.5	0.81	<0.001
Fat depth (mm)	17.94	19.34	16.86	20.42	5.01	0.01	<0.001
Loin depth (mm)	70.68	68.15	69.52	69.32	6.14	0.08	0.77
Lean meat (%)	55.8	55.3	56.0	55.1	1.7	<0.01	<0.001
Yield (%)	75.1	75.1	75.0	75.1	3.1	0.98	0.75
Grade premium (US$)	6.67	6.14	6.35	6.46	1.78	0.61	0.60
Sort loss (US$)	-1.70	-2.06	-1.21	-2.55	2.60	0.21	<0.001

Data are the LSMeans, RSD=Residual standard deviation

No significant interactions between treatment and sex occurred

Table 6-9. Fatty acid concentration (as % of total fatty acids) in sow serum during lactation, colostrum and milk for sows fed either with (mLCPUFA) or without (CON) a supplement rich in marine-oil based n-3 LCPUFA

	Sow serum				Colostrum				Milk			
	CON	mLCPUFA	RSD	P-value	CON	mLCPUFA	RSD	P-value	CON	mLCPUFA	RSD	P-value
n	12	16			12	13			14	17		
unknown	2.70	2.76	0.73	0.81	1.43	1.26	0.26	0.13	ND [a]	ND [a]	.	.
SFA	23.61	23.46	1.97	0.85	30.58	31.07	2.47	0.62	47.57	46.50	4.24	0.49
MUFA	36.37	36.21	3.20	0.89	37.32	37.14	3.52	0.90	39.27	39.66	3.05	0.73
PUFA	37.32	37.57	3.12	0.84	30.68	30.52	3.58	0.92	13.16	13.84	2.14	0.38
C18:2 n-6	26.67	27.21	2.48	0.57	26.53	25.72	3.18	0.53	11.67	12.03	1.80	0.59
C18:3 n-6	0.32	0.32	0.11	0.97	0.36	0.30	0.10	0.13	ND [a]	ND [a]	.	.
C20:3 n-6	0.60	0.56	0.18	0.56	0.38	0.40	0.06	0.55	0.07	0.09	0.07	0.49
C20:4 n-6	6.38	5.41	1.22	=0.05	1.36	1.23	0.19	0.09	0.45	0.42	0.11	0.46
C18:3 n-3	0.45	0.49	0.16	0.52	1.11	1.19	0.18	0.30	0.58	0.58	0.10	0.90
C20:5 n-3	0.28	0.75	0.25	<0.001	ND [a]	0.21	0.08	<0.001	0.02	0.08	0.05	<0.01
C22:5 n-3	1.21	1.42	0.55	0.33	0.11	0.52	0.12	<0.001	0.02	0.17	0.06	<0.001
C22:6 n-3	0.03	0.47	0.17	<0.001	ND [a]	0.26	0.13	<0.001	ND [a]	0.05	0.04	<0.001
Total n-3	1.96	3.13	0.63	<0.001	1.22	2.18	0.41	<0.001	0.62	0.89	0.16	<0.001
Total n-6	33.96	33.50	2.81	0.67	28.63	27.65	3.31	0.46	12.19	12.53	1.92	0.62
n-6:n-3 ratio	18.27	11.59	4.44	<0.001	24.24	13.29	4.21	<0.001	19.72	14.81	3.90	<0.01

Data are the LSMeans, RSD=residual standard deviation, SFA=saturated fatty acids, MUFA=mono-unsaturated fatty acids, PUFA=poly-unsaturated fatty acids, [a] ND=not detectable

Table 6-10. Fatty acid concentration (in mg/l) in sow serum during lactation, colostrum and milk for sows fed either with (mLCPUFA) or without (CON) a supplement rich in marine-oil based n-3 LCPUFA

	Sow serum				Colostrum				Milk			
	CON	mLCPUFA	RSD	P-value	CON	mLCPUFA	RSD	P-value	CON	mLCPUFA	RSD	P-value
n	12	16			12	13			14	17		
unknown	64.6	57.1	51.1	0.70	273.7	266.8	111.5	0.88	ND [a]	ND [a]	.	.
SFA	520.7	446.8	307.3	0.53	5527.5	6226.8	2211.9	0.44	$41.8x10^3$	$38.2x10^3$	$7.7x10^3$	0.20
MUFA	841.7	721.1	607.3	0.61	7161.0	7769.3	2923.4	0.61	$34.8\ x10^3$	$33.1\ x10^3$	$8.5\ x10^3$	0.57
PUFA	840.6	731.8	548.2	0.61	5682.8	6448.5	2137.3	0.38	$11.8\ x10^3$	$11.6\ x10^3$	$3.8\ x10^3$	0.86
C18:2 n-6	595.2	531.1	382.1	0.66	4903.1	5429.9	1805.9	0.47	$10.5\ x10^3$	$10.1\ x10^3$	$3.2\ x10^3$	0.71
C18:3 n-6	7.5	6.6	6.0	0.70	65.5	65.0	28.3	0.96	ND [a]	ND [a]	.	.
C20:3 n-6	13.2	11.2	9.7	0.61	69.3	83.1	25.4	0.19	68.3	77.0	64.6	0.71
C20:4 n-6	147.6	102.5	99.0	0.24	258.7	259.1	103.7	0.99	403.5	348.6	150.6	0.32
C18:3 n-3	10.6	9.7	7.9	0.76	211.6	251.9	91.2	0.28	512.7	482.6	135.8	0.54
C20:5 n-3	5.5	14.5	7.7	<0.01	ND [a]	45.9	20.4	<0.001	17.8	68.2	43.3	<0.01
C22:5 n-3	25.2	25.4	14.2	0.97	21.1	109.6	34.5	<0.001	19.1	145.9	63.6	<0.001
C22:6 n-3	1.2	10.2	5.4	<0.001	ND [a]	56.4	30.4	<0.001	ND [a]	51.3	38.9	=0.001
Total n-3	42.5	59.8	30.9	0.15	232.6	463.8	153.4	=0.001	549.9	748.1	230.0	<0.05
Total n-6	763.4	651.4	492.3	0.56	5296.7	5837.1	1946.0	0.49	$11.0\ x10^3$	$10.5\ x10^3$	$3.4\ x10^3$	0.70
n-6:n-3 ratio	18.3	11.6	4.4	<0.001	24.2	13.3	4.2	<0.001	19.7	14.8	3.9	=0.001

Data are the LSMeans, RSD=residual standard deviation, SFA=saturated fatty acids, MUFA=mono-unsaturated fatty acids, PUFA=poly-unsaturated fatty acids, [a] ND=not detectable

Table 6-11. Fatty acid concentration (as % of total fatty acids) in brain, liver and Semitendinosus muscle tissues from stillborn piglets, born to sows fed either with (mLCPUFA) or without (CON) a supplement rich in marine-oil based n-3 LCPUFA

	Brain				Liver				Muscle			
	CON	mLCPUFA	RSD	P-value	CON	mLCPUFA	RSD	P-value	CON	mLCPUFA	RSD	P-value
n	13	18			12	14			10	17		
unknown	10.52	7.63	1.14	<0.001	4.90	3.87	0.84	<0.01	7.38	6.59	1.11	0.09
SFA	37.29	37.00	4.43	0.86	38.30	39.47	2.67	0.27	40.18	40.12	1.98	0.93
MUFA	29.16	29.44	4.83	0.87	39.64	34.90	5.13	<0.05	34.66	34.95	2.24	0.75
PUFA	23.00	25.86	2.74	<0.01	16.70	21.34	5.58	<0.05	17.78	18.35	3.27	0.67
C18:2 n-6	0.26	0.40	0.42	0.38	4.83	5.43	3.40	0.66	7.45	7.42	3.04	0.98
C18:3 n-6	ND [a]	ND [a]	.	.	0.19	0.18	0.14	0.80	0.27	0.26	0.04	0.80
C20:3 n-6	0.25	0.29	0.07	0.17	0.48	0.63	0.13	<0.01	0.64	0.69	0.12	0.28
C20:4 n-6	9.98	9.14	3.22	0.48	8.48	10.22	2.61	0.10	6.75	6.60	1.51	0.81
C18:3 n-3	1.04	1.42	3.36	0.76	0.23	0.15	0.07	=0.01	0.18	0.18	0.03	0.97
C20:5 n-3	0.48	0.41	0.14	0.24	0.03	0.16	0.12	=0.01	0.22	0.30	0.06	<0.01
C22:5 n-3	ND [a]	ND [a]	.	.	0.07	0.22	0.19	=0.05	0.29	0.49	0.11	<0.001
C22:6 n-3	6.76	9.26	1.00	<0.001	1.49	3.45	0.30	<0.001	0.49	1.14	0.18	<0.001
Total n-3	7.37	9.88	1.00	<0.001	1.82	3.97	1.06	<0.001	1.18	2.11	0.29	<0.001
Total n-6	11.38	11.04	0.83	0.27	13.98	16.45	4.57	0.18	15.10	14.97	3.12	0.92
n-6:n-3 ratio	1.55	1.13	0.14	<0.001	7.93	4.38	1.77	<0.001	12.82	7.33	2.31	<0.001

Data are the LSMeans, RSD=residual standard deviation, SFA=saturated fatty acids, MUFA=mono-unsaturated fatty acids, PUFA=poly-unsaturated fatty acids, [a] ND=not detectable

Table 6-12. Fatty acid concentration (in mg/kg) of brain, liver and Semitendinosus muscle tissues from stillborn piglets, born to sows fed either with (mLCPUFA) or without (CON) a supplement rich in marine-oil based n-3 LCPUFA

	Brain				Liver				Muscle			
	CON	mLCPUFA	RSD	P-value	CON	mLCPUFA	RSD	P-value	CON	mLCPUFA	RSD	P-value
n	13	18			12	14			10	17		
unknown	1597.4	1150.2	469.7	0.01	435.0	332.3	125.5	<0.05	715.8	543.8	145.7	<0.01
SFA	5505.4	5283.4	1456.0	0.68	3521.9	3431.3	1228.0	0.85	3917.9	3384.3	912.5	0.15
MUFA	4460.2	4549.3	1696.2	0.89	3756.0	3144.4	459.7	0.30	3403.9	2979.5	916.6	0.26
PUFA	3428.9	3909.4	1417.5	0.36	1556.6	1793.7	791.4	0.45	1732.1	1549.9	557.1	0.42
C18:2 n-6	37.7	67.6	91.6	0.38	521.9	472.1	470.8	0.79	729.6	650.4	444.5	0.66
C18:3 n-6	ND [a]	ND [a]	.	.	20.9	15.2	18.7	0.45	26.2	22.0	6.6	0.13
C20:3 n-6	39.1	46.0	14.6	0.20	44.2	54.7	23.6	0.27	62.5	56.6	13.9	0.30
C20:4 n-6	1623.4	1521.4	482.7	0.57	728.7	847.5	236.3	0.21	652.8	537.3	126.3	<0.05
C18:3 n-3	24.3	29.9	17.2	0.38	20.6	13.5	9.7	0.08	18.2	15.7	5.7	0.28
C20:5 n-3	72.1	65.6	31.1	0.57	2.4	12.5	9.8	<0.05	22.1	24.7	7.1	0.36
C22:5 n-3	ND [a]	ND [a]	.	.	8.8	19.9	19.6	0.16	28.3	41.5	14.3	<0.05
C22:6 n-3	998.0	1379.3	414.7	<0.05	127.0	283.8	81.7	<0.001	47.3	96.0	25.5	<0.001
Total n-3	1094.4	1474.8	441.4	<0.05	1158.8	329.7	102.5	<0.001	115.8	177.9	46.6	<0.01
Total n-6	1723.7	1664.7	529.8	0.76	1315.8	1389.5	678.5	0.78	1471.1	1266.3	498.8	0.31
n-6:n-3 ratio	1.6	1.2	0.1	<0.001	7.9	4.4	1.8	<0.001	12.8	7.3	2.3	<0.001

Data are the LSMeans, RSD=residual standard deviation, SFA=saturated fatty acids, MUFA=mono-unsaturated fatty acids, PUFA=poly-unsaturated fatty acids, [a] ND=not detectable

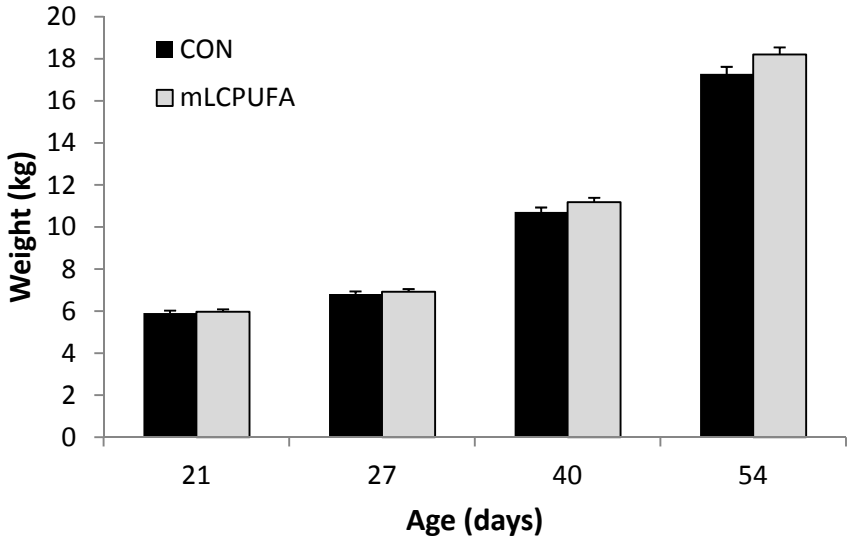

Figure 6-1. Body weight during the nursery period for litters from sows fed either with (mLCPUFA; n=46 pens) or without (CON; n=45 pens) diets enriched with marine-oil based n-3 LCPUFAs. Overall effect of treatment, P= 0.13: effect of time, P < 0.001: interaction between treatment and time, P = 0.18. Data are the LSMeans + Standard Error

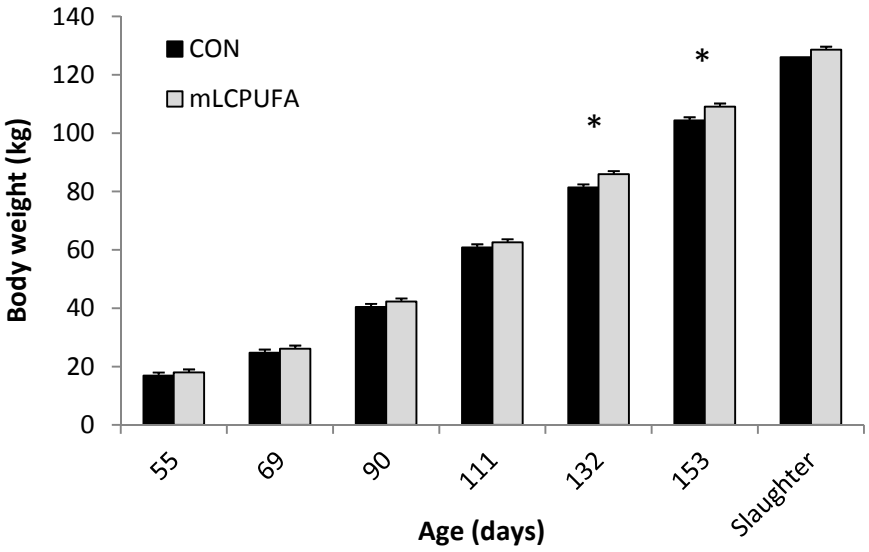

Figure 6-2. Body weight change during the grow-finish period in Barn A littermates from sows fed diets either with (mLCPUFA; n=47 pens) or without (CON; n=48 pens) enrichment with marine-oil based n-3 LCPUFAs. There was a significant interaction between treatment and time ($P < 0.01$).

* denotes ages at which treatment means differed, $P < 0.01$.

Data are the LSMeans + Standard Error

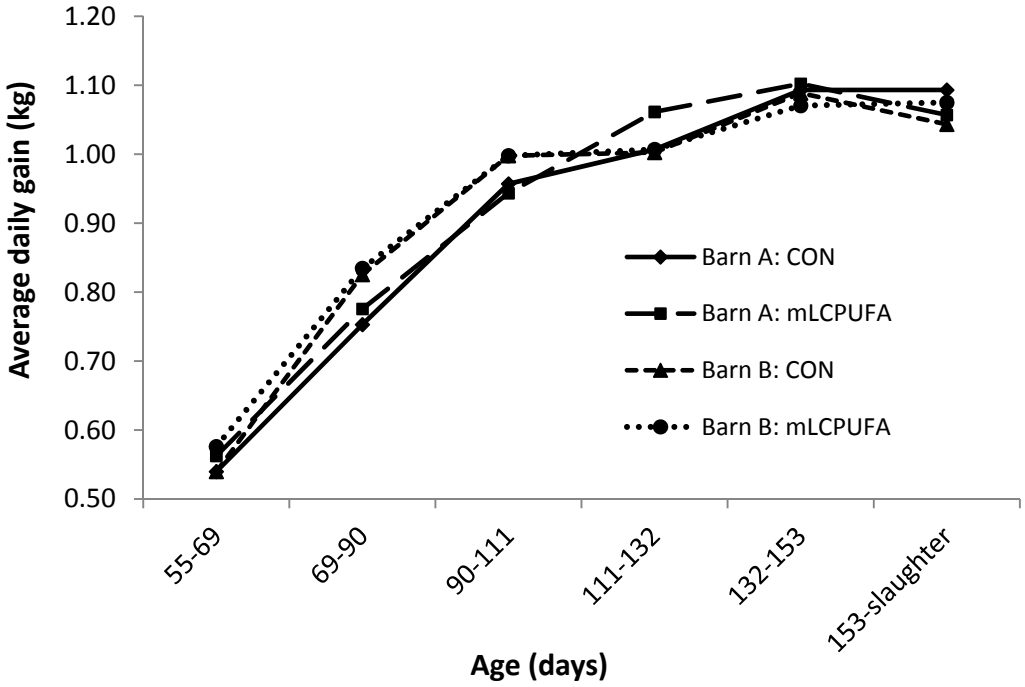

Figure 6-3. Average daily gain during the grow-finish period in barn A and B for littermates from sows fed diets either with (mLCPUFA) or without (CON) enrichment with marine-oil based n-3 LCPUFAs. There was no significant treatment effect in either barn (P=0.16 for barn A and P=0.74 for barn B) and no interaction between treatment and time. Data are the means. N=48 for CON and mLCPUFA in barn A and n=24 for CON and mLCPUFA in barn B

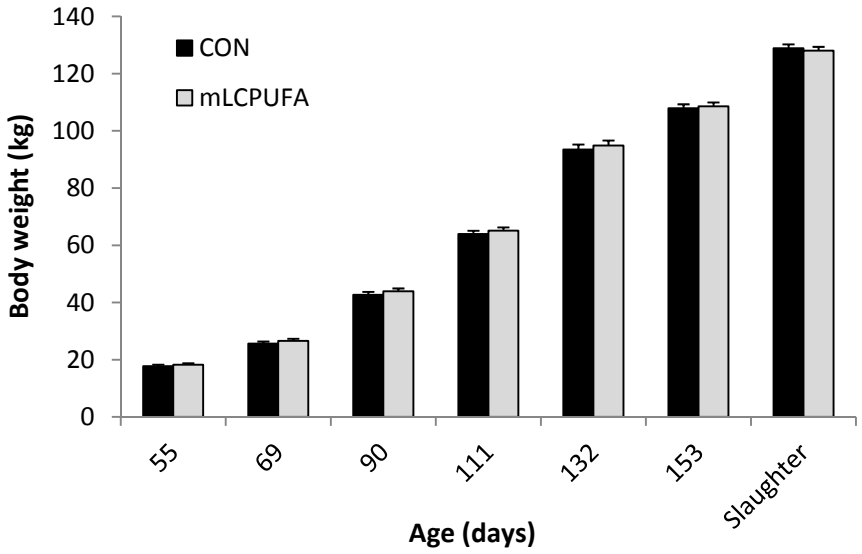

Figure 6-4. Body weight change during the grow-finish period in barn B littermates from sows fed diets either with (mLCPUFA; n=24 pens) or without (CON; n=24 pens) enrichment with marine-oil based n-3 LCPUFAs. Overall effect of treatment, P=0.57: effect of time, P < 0.001: interaction between treatment and time, P = 0.98.
Data are the LSMeans + Standard Error

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Chapter 7: General discussion and conclusions

In this Chapter, the findings from Chapters 3 to 6 are discussed and compared with existing literature, to draw final conclusions, and to give practical recommendations. In addition, questions raised by the combined findings and potential future research are discussed.

7.1 The origin of low litter birth weight

The principal concept driving this research was that changing patterns of pre-natal loss of embryos in contemporary commercial sow populations limits placental development, resulting in IUGR, and that IUGR is a characteristic of entire low average birth weight (LBW) litters. Therefore, it was expected that the LBW litters described in this thesis would show the benchmarks of IUGR, measured as a lower placental weight and the occurrence of brain sparing. Indeed, in both the studies described in Chapters 4 and 5, LBW litters had lower placental weight and, as measured in representative stillborn littermates, a higher brain:liver weight ratio than medium (MBW) or high birth weight (HBW) litters. Table 7-1 compares the information for the different birth weight categories of both data sets. The number of total pigs born in a litter was very similar between studies and between birth weight categories. This reflects the criteria used to select sow litters for the LBW, MBW or HBW categories, which were designed to exclude the effects that very large or small litters have on birth weight, and instead focus on the large variation in litter average birth weight shown by sows that produce litters of a more normal size (between 9 and 16 total born). Litters between 9 and 16 total pigs born were categorized as LBW or HBW if the litter average birth weight was more than one standard deviation below or above the population litter size mean respectively. Litter average birth weight and placental weight data were very similar between data sets, although there were some differences in the percentage of pigs born alive and stillborns per litter. In the second study, LBW litters tended to have a lower percentage of live born and a higher percentage of stillborn pigs per litter and the number of mummies was significantly higher in LBW litters. Experimentally, it is easy to miss small mummies when they are expelled. In the trial described in Chapter 5, there were fewer sows on trial than in Chapter 4. Moreover, during the days that most sows farrowed, personnel were in the barn at approximately 11 pm in the evening to collect colostrum samples. Therefore,

it is likely that more mummies were found in the trial described in Chapter 5, and that information on the number of mummies is more accurate in this than in the earlier study. A higher number of mummies indicates higher fetal losses in LBW litters, which is consistent with the view that low litter average birth weight is due to intra-uterine crowding in early gestation, followed by a wave of fetal loss between day 30 and 50 of gestation.

Table 7-1 also shows the comparison of necropsied piglets between the two studies described in Chapters 4 and 5. One important question to discuss is the extent to which necropsy of stillborn littermates after farrowing can be considered to be representative of the developmental state of the entire litter. In Chapter 4, only true stillborn pigs (did not breathe following parturition) with an individual birth weight within 0.5 kg from the litter average birth weight were included, thereby eliminating small piglets from analysis. Assuming that stillbirth is an event occurring during the farrowing process, and that it is a relatively random process in relation to pig size and viability, the selected stillborn pigs are likely to be representative of the developmental state of the litter. In Chapter 5, all necropsied pigs (50% true stillborns and 50% pigs that died shortly after birth) were taken into account for the analysis, although runts (defined as being > 2St.Dev. from the litter mean birth weight) were not necropsied in either study. Given that the necropsied pigs in Chapter 5 did not need to fall within 0.5 kg of the litter average birth weight, the individual birth weight of necropsied pigs was lower than in Chapter 4, regardless of birth weight phenotype. The lower individual birth weights in Chapter 5 vs. 4 is also reflected by the lower organ weights, and higher brain:organ weight ratios. However, both studies clearly show the association between a lower litter average birth weight phenotype and higher brain:organ weight ratios as a good measure of IUGR (Cooper, 1975). Therefore, data derived from the necropsy of stillborn pigs allows one to conclude that the litters classified as low birth weight in our studies had undergone IUGR. Again, this is consistent with the view that low litter birth weight is due to intra-uterine crowding in early gestation, which affects placental weight at day 30 of gestation, resulting in IUGR.

Intrauterine crowding is believed to be due to high ovulation rates in higher parity sows, followed by decent to good embryonic survival to day 30 of gestation. Although the ovulation rate and embryonic survival of sows on trial is not known, some sows that

repeatedly gave birth to litters with a low or a high average birth weight (see information about repeatability of birth weight below) were followed in the next pregnancy, and were killed at day 20 of gestation. There were 9 sows with a predicted LBW phenotype and 6 sows with a predicted HBW phenotype euthanized, and all of these sows had been on the control diet in the final study. Mean ovulation rates of sows with predicted LBW and HBW phenotypes were 31.1 and 26.3, respectively. Although the number of sows that were euthanized was very limited, the higher ovulation rate in the LBW group would suggest that the low litter birth weight phenotype is indeed connected to higher ovulation rates, and that sows repeatedly have high ovulation rates in different parities. Interestingly, the same outcome was reported in recent preliminary results from small populations of repeatedly LBW and HBW sows studied in a Canadian commercial population (Foxcroft, 2012).

All these results strongly support the hypothesis that, independent of litter size born, low litter birth weight in a substantial population of mature commercial sows is due to a cascade of pre-natal events, namely: 1) high ovulation rates (>25 ovulations) followed by decent to good embryonic survival, 2) intrauterine crowding in early gestation, 3) limited placental development from day 30 of gestation onwards, and 4) measurable effects on fetal development by day 50 of gestation onwards.

7.2 Effect of litter birth weight phenotype on testicular development, growth performance and carcass quality

Low litter birth weight was hypothesized to result in reduced testicular development. Indeed, low birth weight males had lower absolute numbers of germ cells, Sertoli cells and Leydig cells than high birth weight males, but the numbers of cells per gram of testes were similar between HW and LW animals. The total number of Sertoli cells achieved, after proliferation during the pre-pubertal period, will determine testicle size in adulthood, as well as the sperm production capacity (Cooke *et al.*, 1992; Hess *et al.*, 1993). In the context of sire-line genetic programs at the multiplication level, these results have important implications for the effects of litter birth weight phenotype on the lifetime sperm production and libido of prospective AI boars. If mature sows in these sire-line programs show the same repeatability in litter birth weight phenotype as in the

present study (see below), selection of potential AI boars from high birth weight litters would be predictive of better lifetime productivity in the boar stud.

It was also hypothesized that low birth weight litters would not grow as fast as high birth weight litters, and would have lower carcass quality. Indeed, data in Chapter 4 demonstrated that low birth weight litters had lower ADG throughout lactation and most of the nursery and grow-finish phase, and the difference in body weight between litters with low and high average birth weight increased over time. This resulted in low birth weight litters needing 9 more days to reach the same slaughter weight as high birth weight litters.

As intrauterine crowding affects all piglets in a litter (Bérard *et al*., 2010), it was hypothesized that a piglet with an individual birth weight of 1.5 kg born in a low birth weight litter would have a lower growth potential compared to a piglet with an individual birth weight of 1.5 kg born in a high birth weight litter. However, when looking at individual piglets with a birth weight between 1.4 and 1.6 kg, piglets from low birth weight litters had a higher ADG during lactation than piglets from HBW litters. As discussed in Chapter 4, competition between littermates for milk resources probably played a role in this result. Once the pigs were placed in nursery pens, the ADG was similar between LBW and HBW pigs between 1.4 and 1.6 kg birth weight. This result does not support our hypothesis that lower growth potential is a characteristic of entire LBW litters. However, it is still possible that pigs from LBW litters end up with more fat and less lean tissue when slaughtered at the same body weight as HBW pigs, due to the lower numbers of muscle fibers in LBW pigs (Rehfeldt and Kuhn, 2006; Tristán *et al*., 2009; Bérard *et al*., 2010).

An effect of litter birth weight phenotype on carcass quality was not clearly established. Unfortunately, the number of replicates in the HBW group was low due to issues with identification at the slaughterhouse. As described in Chapter 4, the literature also does not give a consistent effect of birth weight on several carcass traits. Therefore, more research is needed to investigate the effect of litter birth weight phenotype on carcass quality. Table 7-2 compares the carcass data of Chapter 4 with that of Chapter 6. As most of the litters in Chapter 6 fell into the LBW category, and some into the lower limit of the MBW category, it was expected that the results from the control group in Chapter 6

would be comparable with the results from the LBW group in Chapter 4. Although the age at slaughter was fairly similar between studies (1.5 days shorter in Chapter 6 than Chapter 4), live weight and hot carcass weight were considerably higher in Chapter 6 than Chapter 4. This is likely due to the low competition for feed and space for litters in Chapter 6. Interestingly, the loin depth and lean meat percentage were lower, and the fat depth was higher, in litters from Chapter 6 compared to Chapter 4. This suggests that the higher body weight was due to fat deposition, and not lean deposition and may be due to the different growth potential of LBW litters. Bérard *et al*., (2010) showed that intrauterine crowding resulted in pigs with lower secondary and total myofibers at birth and generally, myofibers grow more rapidly after birth when the number of fibers is low (summarized by Rehfeldt *et al*., 2000). Therefore, if LBW litters indeed underwent intrauterine crowding, then the pigs in these litters would have reached the plateau of fiber growth earlier than pigs of HBW litters. Any additional nutritional energy would thus be used to deposit fat, instead of muscle. This thought is in agreement with observations from Rehfeldt and Kuhn (2006) that LBW pigs develop more myofibers of extreme size, probably because they are closer to the plateau of fiber growth at slaughter compared to medium and high birth weight pigs.

7.3 Repeatability of litter birth weight phenotype within sows

Figure 4-5 and Figure 5-3 show the same trend in litter birth weights, with most sows giving birth to a LBW litter, again giving birth to a LBW litter at the next farrowing. Although some LBW phenotype sows gave birth to MBW litters in the subsequent farrowing, none subsequently gave birth to HBW litters. Also most of the sows giving birth to MBW litters in the first farrowing, again gave birth to a MBW litter in the subsequent farrowing, although some sows switched to the LBW or HBW category. Both studies show that most sows (all sows in Chapter 5) giving birth to a HBW litter first, give birth to a MBW litter at the next farrowing. One explanation for this is that the cut-off weights used to classify litters as LBW, MBW or HBW was based on previous farrowing events from the sows on trial. Therefore, sows were older in the studies described in Chapters 4 and 5 than at the time that the cut-off weights were initially calculated. Smit (2007) showed that litter average birth weight increases from parity 1 to 3, and decreases from parity 4 onwards. Therefore, the mean birth weight of the entire population of sows used in our studies was lower than at the time of the calculations for

the cut-off weights and, therefore, more litters fell into the LBW and less into the HBW category over time. This is also the reason why there were so few HBW litters available in the last study, described in Chapters 5 and 6, and why the results of MBW and HBW were pooled for analysis. Despite this overall drop in birth weight in the population, only one sow in Chapter 4 and none of the sows in Chapter 5 moved from the HBW category to the LBW category in consecutive farrowings. This clearly shows that sows giving birth to HBW litters are very unlikely to give birth to a LBW litter next, and vice versa. Together with the observations that the correlation coefficient is reasonably high (r=0.49 for the later farrowings, reported in Chapter 5), it can be concluded that litter average birth weight is repeatable, and thus predictable, within sows. These results are again consistent with analyses based on a population of commercial sows in Canada (J. Patterson, personal communication and unpublished data).

Furthermore, a work visit to Tempel Genetics, a swine genetics company located in Indiana, USA, provided the opportunity to calculate repeatability of birth weight in a dataset collected for about 1.5 years and including information of 1465 purebred sows, of which 278 survived to parity 8. The repeatability of birth weight was calculated for consecutive parities. The correlation coefficients for the first 4 parities were as follows; r=0.68 for parity 1 vs. 2, r=0.74 for parity 2 vs. 3, r=0.72 for parity 3 vs. 4, r=0.65 for parities 1 and 2 vs. 3, r=0.70 for parities 1, 2 and 3 vs. 4, and r=0.71 for parities 2 and 3 vs. 4. All correlation coefficients were highly significant ($P<0.001$). These correlation coefficients were much higher than those observed in our studies from Chapters 4 and 5, where the highest correlation coefficient was 0.49 for farrowing event 3 vs. 4, and r=0.50 for farrowings 1, 2 and 3 vs. 4. This suggests that repeatability of litter birth weight is higher in purebred sows than crossbred sows. The data also shows that in purebred sows, having information of more parities did not improve the correlation coefficient, while it did improve somewhat for crossbred sows. This suggests that it will be easier to select on litter birth weight phenotype in purebred populations, as information of only one farrowing event is needed to make reasonable predictions on future litter birth weight phenotype of that sow.

7.4 Research methodologies to investigate effect of birth weight within and between litters

As discussed above, the litters classified as LBW in our studies showed the benchmarks of IUGR, and this is very likely due to intrauterine crowding in early gestation. These results show that the approach of classifying litters as low, medium or high birth weight described in Chapter 4 was effective. However, as mentioned in the previous paragraph, sows were aging throughout the different studies, while the cut-off weights calculated for each birth weight category were determined on a younger population. This resulted in more litters falling in the LBW category and fewer litters falling in the HBW category, especially in the last study (Chapter 5), because the population mean litter weight decreased for the sows on trial. Therefore, cut-off weights should be adjusted for parity in future studies, or cut-off weights should be calculated using the entire sow population on farm, instead of just using sows on trial for that calculation.

Although the LBW litters described in our studies showed the benchmarks of IUGR, it cannot be said with certainty that these litters had undergone intrauterine crowding in early gestation, as nothing was known about the ovulation rate or embryonic survival of these litters. A reliable, but expensive and time-consuming, way to measure this, without euthanizing the sow, is through laparotomy. Laparotomy has been used in previous research for this purpose (Johnson *et al*., 1999; Wilson and Anderson, 2010). A less invasive method of potentially determining the number of viable embryos is by determining circulating estrone sulfate concentrations in the sow. Gaustad-Aas *et al*. (2002) reported this to be a good indicator of litter size *in utero* and Bérard *et al*. (2010) used this method to look at possible differences in the number of viable embryos at day 20 of gestation between 'crowded' and 'less-crowded' sows in their research. Although Gaustad-Aas *et al.* (2002) found a correlation between the number of viable embryos and estrone sulfate concentrations in the sows' blood, it is not clear if a difference, for example, between 15 and 25 embryos can be picked up by this method. A more direct way to study the effects of intrauterine crowding is by manipulating the number of embryos in the uterus. This can be done either 1) through unilateral oviduct ligation, to induce a less crowded uterine environment, which can then be compared to control sows with a relatively crowded uterine environment (Town *et al*., 2004), or 2) by performing unilateral hysterectomy-ovariectomy, to induce a more crowded uterine environment, which can then be compared to a less crowded uterine environment in control sows

(Bérard *et al*., 2010). Possibly the best research to-date is that of Pardo *et al*. (2013) who used both surgical methods to compare sows with extremely crowded versus extremely non-crowded uterine environments. They showed that the 'crowded sows' gave birth to litters with a much lower average birth weight (1.23 kg) compared to 'non-crowded' sows (1.86 kg). This fits with our hypothesis that LBW litters are a result of intrauterine crowding. They also found evidence of brain sparing in the crowded litters compared to the non-crowded litters, similar to our results for low vs. high litter average birth weight (Table 7-1). Moreover, their research showed a smaller muscle cross-sectional area in the *Semitendinosus* muscle and a delayed muscle development in pigs from crowded litters. Unfortunately, this study did not follow litters post-natally to study the effect of intrauterine crowding on post-natal growth performance and carcass quality. I would therefore suggest a follow-up study with the same research design, but that follows the litters to market and that measures body weight at different time points and carcass quality.

Most research to study the effects of birth weight on growth performance and carcass quality has looked at the effect of individual birth weight of pigs within litters, or within populations, rather than the effects of litter average birth weight within a population of litters. Some researchers have chosen pigs in certain birth weight categories regardless of the litters they came from (Quiniou *et al*., 2002; Alvarenga *et al*., 2012) and this could lead to a higher variation in the studied parameters, due to the fact that litter effects are not taken into account. Other researchers used comparisons of low, medium and high birth weight pigs within the same litters (Gondret *et al*., 2006; Rehfeldt and Kuhn, 2006; Beaulieu *et al*., 2010). Although this removes the litter effect, it is important to note that the litters used with this methodology may be different litters than the LBW and HBW litters used in our studies. For example, Beaulieu *et al*. (2010) selected litters that had pigs that fell into 4 different birth weight quartiles; 0.80-1.20 kg, 1.25-1.45 kg, 1.50-1.70 kg and 1.75-2.50 kg. Interestingly, when analyzing all litters used in the study described in Chapters 5 and 6, only five litters in the LBW category had piglets falling in all four birth weight quartiles adopted by Beaulieu *et al*. (2010). Figure 7-1 shows that although HBW litters often do have pigs in all four birth weight quartiles, the LBW litters lack pigs in the highest, and sometimes even in the highest two, birth weight quartiles. Thus, most of the pigs in the LBW litters in our study fell into the lowest two birth weight quartiles, and results from the study of our LBW litters should therefore be compared to

the results of those birth weight quartiles. Nevertheless, it should also be appreciated that the individual pigs falling into the lower individual birth weight quartiles in the litters studied by Beaulieu *et al*. (2010) most likely did not experience the intrauterine crowding predicted to result in the LBW phenotype studied in the experiments reported in this thesis. The results of our studies of LBW litters might, therefore, be expected to show more extreme effects of birth weight on growth performance and carcass quality. However, direct comparisons between studies is further confounded by different ages and weights at slaughter, which makes comparisons of ADG in the finisher period, and days to market difficult to compare.

To further elucidate the effect of individual birth weight versus litter average birth weight on post-natal growth performance, research should be carried out on a subset of pigs with individual birth weights between 1.4 and 1.6 kg that would be followed from birth to slaughter. Most low and high birth weight litters have a few pigs falling in this birth weight range. Chapter 4 included a retrospective analysis of this subset. However, the problem in the study of Chapter 4 is that these piglets were raised in their respective litters, which gave the heavier pigs of the low birth weight litters an advantage in competition for colostrum and milk, while the pigs with the same individual birth weight were the lowest weight pigs in the HBW litters. As this could explain the higher ADG found in lactation for pigs from the low birth weight vs. the high birth weight group in Chapter 4, in future research, equal weight pigs from HBW and LBW litters should be paired and should be cross-fostered to another nurse sow. This way, the rearing conditions in lactation are the same for both pigs, and any differences in growth rates can be attributed to their different pre-natal environments. Moreover, individual carcass data should be obtained on those pigs. In Chapter 4, ADG was similar for the subset of pigs in nursery and grow-finish, but as no individual carcass data was available, we could not determine if growth rate was due to lean tissue, or fat, accumulation. As described above, we hypothesize that pigs from low birth weight litters reach the plateau of fiber growth earlier, and that this will result in fatter carcasses. It would be very interesting to see if this indeed is the case when looking at pigs with the same individual birth weight.

7.5 Effect of maternal marine-oil based n-3 LCPUFA supplementation on litter and subsequent sow reproductive performance

Data in Chapters 3 and 6 showed that both the EPA and DHA in the fish oil supplement fed to gilts or sows were taken up into the bloodstream in gestation, and in late lactation. However, with continued supplementation only DHA increased in embryos at day 30 of the next gestation, consistent with the results of Brazle *et al.* (2009). This confirms that either DHA is selectively taken up through the placenta into the embryo during early pregnancy or that EPA was also taken up by the embryo but then converted to DHA. Based on the results reported in Chapter 6, the higher concentration of DHA in tissues of stillborn piglets again shows the uptake of DHA by the fetus through the placenta. EPA concentrations were also higher in liver and muscle samples from stillborn piglets when sows were fed marine-oil based n-3 LCPUFA, showing that EPA must be able to cross the placenta later in gestation. These results confirm the belief that piglets can benefit from marine-oil based n-3 LCPUFA supplementation to the sow prenatally, when developing embryos have access to DHA and later on also EPA. Data presented in Chapter 6 also showed that EPA and DHA were elevated in colostrum and milk from marine-oil based n-3 LCPUFA supplemented sows, in agreement with previous findings (Taugbol *et al.*, 1993; Rooke *et al.*, 2000; Leonard *et al.,* 2010). Thus, postnatally, piglets can also benefit from maternal marine-oil based n-3 LCPUFA supplementation when litters consume colostrum and milk containing elevated concentrations of EPA and DHA.

It was hypothesized that marine-oil based n-3 LCPUFA supplementation to gilts from day 60 of gestation and during lactation would improve subsequent reproductive performance. The lactational feed restriction model described in Chapters 1 and 3 had been used as a background challenge against which to determine beneficial effects of marine-oil based n-3 LCPUFA supplementation to gilts during their first lactation on subsequent reproduction. Second parity sows often show suboptimal litter sizes and/or farrowing rates, known as the 'second parity dip'. On a population basis, this has recently been associated with high body weight loss during first lactation (Schenkel et al., 2010; Hoving, 2012). A comprehensive series of more basic studies have demonstrated the metabolic and biological components of the 'second parity dip' (see reviews of Foxcroft, 1992 and Quesnel, 2009). Supplementation of gilts and primiparous sows with marine-oil based n-3 LCPUFA did not change weaning-to-estrus interval, and did not improve embryonic survival or embryo quality at day 30 of gestation. Only CL were heavier in

marine-oil based n-3 LCPUFA supplemented sows, and higher levels of EPA and DHA were found in the CL of those sows. However, the higher CL weight was not linked to plasma progesterone concentrations 60-72 hours after ovulation. The influence of a higher CL weight and higher concentrations of EPA and DHA on reproductive performance later in gestation is not known, but is likely minor. Therefore, based on the study reported here, it can be concluded that maternal marine-oil based n-3 LCPUFA supplementation did not improve reproductive performance of primiparous sows, and that marine-oil based n-3 LCPUFA supplementation is not a good strategy to overcome the negative effects of lactational catabolism, and to prevent the second parity dip. However, compared to data reported from many commercial sow herds, the impacts of lactational catabolism in our study were minimal and the control catabolic sows had exceptional reproductive performance. In herds and circumstances in which the 'second parity dip' is more evident, marine-oil based n-3 LCPUFA supplementation may still be beneficial, and more controlled experiments in different production environments are needed.

It was also hypothesized that marine-oil based n-3 LCPUFA supplementation to gilts from day 60 of gestation and during lactation would increase growth performance of their offspring until the end of the nursery phase. Indeed, body weight tended to be higher until the end of the nursery phase for offspring from marine-oil based n-3 LCPUFA supplemented sows compared to control sows. Surprisingly, no research has been done to-date to look at the effect of maternal n-3 LCPUFA supplementation on growth performance to slaughter, and to investigate carcass quality. In our study described in Chapter 6, only low birth weight litters were followed from birth to slaughter, and carcass traits were only measured in low birth weight litters. Further research should be done to investigate the effect of maternal marine-oil based n-3 LCPUFA supplementation on growth performance to slaughter and carcass quality in the entire population of litters.

7.6 Effect of maternal n-3 LCPUFA supplementation on litter size

Although more information has become available about the mechanisms of action of n-3 LCPUFA in the body, a number of questions remain to be answered. In particular, the different results with regards to litter size seem to stand out. The higher incidence of stillborns seen by Eastwood *et al*. (2011) could possibly be explained by the action of $PGF2_{\alpha}$. The precursor to $PGF2_{\alpha}$ is the n-6 LCPUFA AA. As was seen in Chapters 3 and

6, and reported by Rooke *et al*. (2000), AA was decreased in the sow's blood circulation when fed n-3 LCPUFA. A lower amount of AA in the blood could lead to a lower production of $PGF2_{\alpha}$, an important luteolytic factor, which could increase the interval between piglets farrowing, and in turn increase the chance of stillborns. Why this increase in number of stillborns was not seen in many other trials, including those described in this thesis, is unclear.

The research of Webel *et al*. (2003) and Spencer *et al*. (2004) associated treatment of sows with n-3 LCPUFA with increases in the total number of pigs born and Webel *et al*. (2004) showed that early embryonic survival was higher in the n-3 LCPUFA fed sows. However, as discussed in Chapter 3, embryonic survival of their control sows was low. It is thus possible that the limiting factor for litter size in the trials of Webel *et al*. (2003) and Spencer *et al*. (2004) was early embryonic survival. As already suggested in Chapter 3, it is possible that the fish oil product used (Fertilium/Gromega/Sow Fat Pack 10) only improves embryonic survival if the survival is initially poor. Although this would explain why we didn't see a difference in litter size in Chapter 3, where embryonic survival of control sows was already high, the decrease in litter size seen in Chapter 5 cannot be explained on this basis and was not expected. More research is needed to investigate the effect of maternal n-3 LCPUFA supplementation on litter size. Factors like the timing and amount of n-3 LCPUFA supplementation, as well as the n-6:n-3 LCPUFA ratio, should be considered in these studies.

7.7 Maternal marine-oil based n-3 LCPUFA supplementation and litter birth weight phenotype

Considering the lower growth rate in LBW litters found in Chapter 4, and the increased growth potential of pigs born from sows supplemented with marine-oil based n-3 LCPUFA found in Chapter 3, we reasoned that feeding marine-oil based n-3 LCPUFA to sows that give birth to LBW litters would increase the growth rate of their offspring, thereby decreasing the gap in growth rate between LBW and HBW litters. Therefore, to specifically look at the effect of marine-oil based n-3 LCPUFA supplementation on growth rate of LBW litters, the study described in Chapters 5 and 6 was completed. Maternal marine-oil based n-3 LCPUFA supplementation was hypothesized to increase growth performance of LBW litters and improve carcass quality. In contrast, the results

showed that maternal marine-oil based n-3 LCPUFA supplementation only increased body weight at weaning and ADG in lactation in pigs from MBW or HBW litters, and did not improve growth performance until weaning in pigs from LBW litters. When following the LBW litters in the nursery and grow-finish phase, ADG, ADFI and feed utilization efficiency were not affected by maternal marine-oil based n-3 LCPUFA supplementation. Furthermore, body weight was only found to be higher in the second half of the grow-finish phase in LBW pigs from sows fed marine-oil based n-3 LCPUFA, when pigs were individually penned and competition for feed or space was minimal. However, pigs in a commercial setting will always have some competition for feed or space, and positive effects of maternal marine-oil based n-3 LCPUFA supplementation on growth performance of LBW litters in a commercial setting are, therefore, not expected.

The study also hoped to establish a positive effect of maternal marine-oil based n-3 LCPUFA enrichment on offspring carcass quality, offsetting the possible negative effects of LBW on carcass quality. Instead, maternal marine-oil based n-3 LCPUFA enrichment increased fat depth, tended to decrease loin depth and decreased lean meat percentage. Thus, the marine-oil based n-3 LCPUFA enrichment of sows resulted in a negative effect on exactly the same parameters that were also negatively affected by low birth weight.

Maternal marine-oil based n-3 LCPUFA supplementation did not result in differences in brain:liver weight ratio, or placental weight in litters between 9 and 16 total pigs born, suggesting that marine-oil based n-3 LCPUFA enrichment does not affect the processes related to IUGR. The fact that there was no interaction between treatment and birth weight category for placental weight, or any of the tissue weights and brain:tissue weight ratios, also supports this suggestion. Possibly, the negative effects of IUGR on muscle development then limits the potential for a product like fish oil rich in n-3 LCPUFAs to produce positive effects to market weight. If LBW pigs indeed reach their plateau of lean growth potential around the time they reach market weight, as suggested above, then it makes sense that nutritional interventions after birth may not be capable of exerting an effect on lean growth performance. An effect on fat deposition, as seen in our study, would however still be possible in the absence of effects on muscle growth.

Overall, it can be concluded that maternal marine-oil based n-3 LCPUFA supplementation is not a good strategy to overcome the negative effects of a LBW phenotype.

7.8 Effect of litter birth weight phenotype and maternal marine-oil based n-3 LCPUFA supplementation on immune function

The LBW litters described in Chapters 4 and 5 showed the benchmarks of IUGR, having a lower placental weight and a higher brain:liver weight ratio than MBW or HBW litters. Furthermore, the brain:thymus and brain:spleen weight ratios were also increased in low birth weight litters. Spleen weight was sex-dependent; only males from LBW litters had lower spleen weight than pigs from MHBW litters. A lower spleen weight was also detected by Town *et al*. (2005) in litters that had been relatively crowded *in utero* in early gestation, and Harding *et al.* (2006) showed that spleen:brain and thymus:brain weight ratio were highly correlated with birth weight. Both the spleen and thymus play important roles in the immune system. The main function of the thymus gland is to produce mature T cells. Immature cells, produced at the bone marrow, migrate to the thymus, where maturation takes place. The spleen is composed of T-cells, B-cells, natural killer cells, macrophages, dendritic cells and red blood cells and acts as an immunologic filter of the blood, entrapping antigens from the blood passing through the spleen. When the macrophages and dendritic cells bring antigens to the spleen via the bloodstream, the B cells in the spleen are activated and produce large levels of antibodies (Delves et al., 2006). A decrease in the weight of the thymus and spleen could, therefore, have functional consequences for the immune system of low birth weight pigs. However, to-date, little is known about these possible associations.

Although the spleen and thymus weight were not different between pigs born to sows fed with or without marine-oil based n-3 LCPUFA, n-3 LCPUFA supplementation has also been suggested to have an effect on immune response, because of its immunomodulatory properties (Calder, 2007). Additionally, they have been shown to alleviate inflammatory conditions in humans like rheumatoid arthritis and inflammatory bowel disease (Miles and Calder, 2012; Ruggiero *et al*., 2009). However, it is not known whether the negative regulation of the immune system by n-3 LCPUFA may be detrimental to a pig's ability to mount an effective immune challenge to pathogens.

In association with the studies reported in Chapters 5 and 6 in this thesis, other research in our group (Dr. Jamie Wilkinson, personal communication), indirectly evaluated immune response of pigs near weaning from LBW and HBW litters and born from sows with or without marine-oil based n-3 LCPUFA supplementation (5 sows/litters studied from each of these four categories). Not all HBW litters came from the HBW group described in Chapter 4, due to a shortage of HBW litters. However, all litters had a litter average birth weight above the mean value for the respective litter size. Blood samples were collected from four piglets of each selected litter, two males and two females, that had individual birth weights closest to the litter average birth weight, giving a total of 80 samples for analysis. These samples were immediately shipped for analysis at the University of Alberta, and activation of peripheral blood mononuclear cells (PBMC) by the mitogen concanavalin A was used as an immunoassay for immune responsiveness. Gene expression of two markers of PBMC activation, IL2 and MAD2L1, were used to assess the immune response between LBW and HBW litters, and between litters from sows fed with or without marine-oil based n-3 LCPUFA. Gene expression of IL2 and MAD2L1 was not affected by litter birth weight phenotype or maternal marine-oil based n-3 LCPUFA supplementation. This shows that, at least with this immunoassay, no effects of litter birth weight phenotype or maternal marine-oil based n-3 LCPUFA supplementation on activated immune responses in vitro were found. More research on the effects of litter birth weight phenotype and maternal n-3 LCPUFA supplementation on the immune system in vivo is needed.

Before they acquire active immunity, piglets are entirely dependent on immunoglobulin transfer from sow colostrum to maintain effective passive immunity. IgG is the most important immunoglobulin to boost the piglet's passive immune system. Unfortunately, the results in Chapter 5 on IgG concentration in colostrum were highly variable, and it was not possible to establish any significant differences between LBW and HBW litters, or between litters from sows fed either with or without marine-oil based n-3 LCPUFA. Other research has shown that supplementing sows with n-3 LCPUFA during gestation increased IgG concentrations in colostrum from n-3 LCPUFA supplemented sows, using the same fish oil product as in the current trial (Mateo *et al*., 2009). Moreover, Rooke *et al*. (2003) reported enhanced piglet serum IgG concentration at weaning in piglets born to sows fed fish oil. Thus, maternal n-3 LCPUFA supplementation during gestation seems promising in increasing IgG concentration in colostrum. The fact that Leonard *et al*.

(2010) did not show higher IgG concentrations in colostrum from sows fed fish oil from 109 days of gestation onwards suggests that a longer period of supplementation before parturition is necessary to see beneficial effects of n-3 LCPUFA supplementation on IgG concentration in colostrum. Further investigation to establish the minimum amount of time of n-3 LCPUFA supplementation needed to see a response in colostral IgG concentration might be useful. Also the effect of litter birth weight phenotype on colostral IgG concentration merits further investigation, as no research to-date has examined this effect. An additional parameter that should be taken into account in these studies is the actual colostrum uptake of pigs, because colostral IgG can only improve the piglet's passive immune system if the piglet receives sufficient colostrum. Various researchers have argued that low birth weight piglets lack the ability to successfully extract colostrum from the teats (Pluske and Williams, 1996; Hoy *et al*., 1997; Milligan *et al*., 2001).

7.9 Practical implications

Although supplementation of marine-oil based n-3 LCPUFA to gilts improved body weight in offspring up until the end of the nursery period, it also increased pre-weaning mortality rate in that study. Supplementation in older sows decreased litter size, including pigs born alive, in our other study. Effect of maternal marine-oil based n-3 LCPUFA supplementation on growth performance of the entire population until slaughter is not known, but our results showed that it did not improve growth performance of LBW litters, and that it negatively affected carcass quality of those litters. Moreover, it did not improve reproductive performance in primiparous sows. Therefore, implementation of marine-oil based n-3 LCPUFA supplementation to gilts and sows on farms as a nutritional intervention seems of limited use.

On the other hand, the negative effects of low litter birth weight on survival rates and growth performance observed in our studies shows that decreasing the number of LBW litters on farms is of utmost importance. In collaboration with Ken Engele, the Swine Innovation Porc manager of Technology Transfer, located at the Prairie Swine Centre (Saskatoon, SK), an economic evaluation of the three birth weight classifications was performed, using information from Chapter 4. The results are shown in Table 7-3 and are in Canadian dollars. In his model, an average hog index price of $165/100 kg dressed carcass weight , and average feed cost of the previous 5 years, ending in October 2012,

were used, with average feed cost per pig at $85.36 for a 115 kg finished pig. Table 7-3 shows comparisons between low and medium, low and high, and medium and high birth weight litters. It demonstrated that if a litter would have been medium birth weight instead of low birth weight, the farmer would have earned $8.64 more for each pig in that litter. So a MBW litter of 12 pigs born alive would mean increased earnings of $103.68 compared to a LBW litter. Considering that 16% of the litters in Chapter 4 fell in the LBW category, it would mean that if all LBW litters were replaced by MBW litters, the earnings would increase by $1.38/pig on a herd basis. Earnings are broken down into several categories in Table 7-3 and the increased earnings in MBW vs. LBW litters is due to the higher number of pigs born alive, lower pre-weaning mortality and the faster growth after weaning. Table 7-3 also shows that a farmer would have received $9.45/pig more for pigs in HBW vs. MBW litters. Considering that 65% of the litters in Chapter 4 fell in the MBW category, the earnings would increase by $6.14/pig on a whole herd basis if all MBW litters would be replaced by HBW litters. When comparing LBW with HBW litters, earnings would be higher by $18.09/pig in HBW vs. LBW litters. If all LBW litters (16% of population) were replaced by HBW litters, this would increase the earnings by $2.89/pig on a whole herd basis. These analyses clearly show that the swine industry should strive to increase litter average birth weight.

As shown by the economic analysis (Table 7-3) the greatest potential to improve earnings on LBW litters is to reduce the pre-weaning mortality rate. It is known that the majority of pre-weaning mortality occurs in the first four days after farrowing (Marchant et al., 2000), and most deaths are due to crushing and starvation. However, these causes are often secondary to the effects of perinatal hypothermia (Edwards, 2002). Therefore, management interventions in the immediate post-farrowing period are an important means to increase survivability of pigs in the LBW litters. Given that litter birth weight phenotype is a repeatable trait in sows, it is possible to move all sows with a predicted low birth weight phenotype into one farrowing room, and make this a ‘high priority room’ for intensive post-natal care. Drying off piglets in that room could prevent against hypothermia, and having personnel check that room frequently can decrease the number of piglets crushed by the sow. Additionally, given the implications of LBW for IUGR and limited development of the gut and immune system, interventions to ensure adequate colostrum intake, like split-suckling and colostrum supplementation, would be indicated.

Another way to possibly improve survivability of pigs in LBW litters is to breed sows with a predicted LBW phenotype to specific boars. Some boars are known to have more robust offspring (Dr. Egbert Knol, personal communication), and selecting AI doses from these boars for mating of sows with a predicted LBW phenotype might thus decrease pre-weaning mortality in this vulnerable population.

Additional earnings can be made in the nursery and grow-finish phases, as shown in Table 7-3. As discussed in Chapter 4, segregated management based on litter birth weight in the nursery and the grow-finish phase seems promising. It is clear that pigs from LBW litters have different nutritional needs than those of HBW litters, and segregated management would help to optimize the feeding of both populations. A lower nutrient profile could be fed to low birth weight litters, because they likely don't have the muscle fibers needed to grow like the high birth weight litters, and as discussed before, additional nutritional energy will just result in higher fat deposition. Another potential advantage of segregated management would be that LBW litters could be marketed differently from the rest of the population. Because LBW pigs likely reach the plateau of lean growth earlier than high birth weight pigs (Rehfeldt and Kuhn, 2006), LBW litters should either be marketed at a lower slaughter weight, or sent to a market demanding higher fat percentages.

An exciting new development, that might become available to the swine industry within the next five years, would help to feed pigs after weaning to their individual needs, even without segregated management. This development consists of precision feeding systems. "Precision feeding involves the use of feeding techniques that allow the right amount of feed with the right composition to be provided at the right time to each pig in the herd." (Pomar and Pomar, 2012). The automated precision feeder recognizes individual pigs in a pen, and measure body weight as soon as the pig enters the feeding station. The feeding station is coupled to a computer system that has information of the past nutrient intake and growth patterns of each pig, and it uses this information to blend two or more premixes to deliver, upon the animal's request, small meals providing the estimated optimal nutrient concentration (Pomar and Pomar, 2012). This system may also be used to make informed decisions on the best timing of slaughter for each pig in the pen. Compared to this system, segregated management may still have one advantage, and that is the more efficient use of barn space. The LBW litters needed 9 more days to reach the

same market weight than HBW litters and this suggests that segregated management of LBW progeny would allow more efficient use of barn turns at the grow-finish stages of production.

Instead of finding management options to deal with low birth weight litters, it would be best to eliminate low birth weight litters in the swine industry altogether. For this purpose, breeding companies should change their focus from increasing litter size to improving litter quality. The litter average birth weight, number of pigs born alive, and survivability to weaning should have high importance in the selection index, while total litter size should have a much lower importance in the selection index. Furthermore, this and subsequent studies have begun to explore the component biological traits associated with a LBW phenotype. As discussed earlier, an exaggerated ovulation rate in mature sows appears to be one important phenotypic trait and initial data indicate associated differences in gene expression in ovarian tissues (Silva, 2012). A limited number of DNA samples obtained for both the sows reported in this thesis and from the study mentioned in Canada have also been submitted for genomic analysis using a commercially available 66k SNP microarray. The intention would be to expand these studies and use association analyses to identify markers for the key component traits driving the LBW phenotype in mature sows. The immediate limitation to this approach is access to a large enough population of sows with a defined BW phenotype, but the repeatability of BW phenotype established in the studies in this thesis and in other sow populations suggests that attention to the capture of litter birth weight data in purebred sow populations has considerable potential to help achieve these goals.

7.10 Strengths and limitations of the research trials

Although some strengths and limitations of the different research trials have been described in earlier chapters, this section presents an overall critique of the research undertaken. The trial described in Chapter 3 was the first to investigate the effects of n-3 LCPUFA supplementation on the 'second parity dip', by using an established lactational feed restriction model to induce catabolism in primiparous sows during late lactation. This feed restriction model was used as a background challenge against which to determine beneficial effects of marine-oil based n-3 LCPUFA supplementation to gilts during their first lactation on subsequent reproduction. However, despite the imposed

feed restriction during lactation, the control sows in this trial had excellent reproductive performance, allowing little opportunity to assess potential beneficial effects of n-3 LCPUFA supplementation on the different reproductive characteristics normally associated with the 'second parity dip' (see Soede *et al.,* 2013). Nevertheless, more subtle repsonses to treatment were observed which may still have value. This trial was the first one to measure effects of maternal marine-oil based n-3 LCPUFA supplementation on the fatty acid profile of embryos on day 30 of gestation, CL weight and CL fatty acid profile. Although the effects of a higher CL weight and changed fatty acid profile on reproductive performance seems minimal, it may have currently unknown effects that merit further research. This was one of a limited number of studies to follow the performance of offspring until the end of the nursery phase, and it would have been even more interesting to follow the offspring to market weight. Unfortunately, the research facility did not allow for this set-up.

Providing the fish oil supplement in this trial as a top dressing to LCPUFA sows, over and above the dietary allowance provided to Control sows created small differences in the total energy intake, which was particularly apparent during the last week of lactation in treated vs. control sows. Using energy intake from feed during the last week of lactation as a covariate in the analyses helped to deal with this problem, but the use of isocaloric diets throughout the trial by providing Control sows with another supplement with similar energy levels as the fish oil supplement would have been a better approach. With unlimited resources, it would also have been interesting to add treatment groups that started n-3 LCPUFA supplementation at different times during gestation, or that would receive a different ratio of the supplement, to take effects of timing and amount of supplementation into account.

The trial described in Chapter 4 was the first to describe the postnatal growth performance of progeny from specific litter average birth weight phenotypes from birth all the way to slaughter. Earlier research has looked at effects of intrauterine crowding on birth weight, brain:liver weight ratios, placental weights and muscle fiber characteristics (Town et al., 2004; Bérard et al., 2010), but none of these have followed the litters after birth. As mentioned earlier in this Chapter, it was not possible to know with certainty what the origin of the litters before birth was, as no information on ovulation rate or embryonic survival was available. Using laparotomy to obtain this information in at least a proportion of the sow, as reported by Dhakal *et al.* (2013) in a comparable study would

have been interesting, but was not feasible in the more commercial type setting of the trial completed in collaboration with JBS United in Indiana. Nonetheless, our results on placental weight and brain:organ weight ratios were similar as those seen by Town et al. (2004) and suggest that LBW litters are a result of intrauterine crowding. Although much research has been done to look at effects of individual birth weight on post-natal growth performance and carcass quality, the effects of the litter average birth weight on these parameters have never been measured before. It was unfortunate that some information of HBW pens was lost in the slaughterhouse, which made definite conclusions about effects of litter birth weight phenotype on carcass quality impossible. It would also have been valuable to get carcass information for individual pigs, particularly with respect to the subset of pigs with an individual birth weight between 1.4 and 1.6 kg, for which post-natal growth performance was analyzed on an individual basis.

The repeatability of litter average birth weight within sows has never been shown before. As mentioned earlier, the cut off weights to determine which litters would fall in the low or high birth weight categories should have been calculated on a whole-herd basis, instead of using the sows on trial. By using data of sows on trial, the information was valid for younger sows only. As overall litter birth weight decreased in higher parity sows, there was a decrease in HBW litters, especially during the last trial described in Chapters 5 and 6.

The trial described in Chapter 5 was the first to describe interactions between maternal marine-oil based n-3 LCPUFA supplementation and litter average birth weight phenotype. It was also the first time that offspring from marine-oil based n-3 LCPUFA supplemented sows were followed to market weight. In the grow-finish phase, pigs were housed in two separate barns and in both barns pigs were housed at a low density (1 to 2 pigs/pen in barn A and 5 to 10 pigs/pen in barn B) compared to commercial housing systems. However, often a treatment will be tested in low density housing systems first. If a treatment effect is found, then the trial will be repeated in commercial housing systems. In our trial, as described in Chapter 6, only LBW litters were selected to be followed to market. It would have been interesting to also follow the litters from MHBW litters to market, but the research facility did not have enough space in their low density housing barns to facilitate this. Although very few effects of maternal n-3 LCPUFA supplementation were found in LBW litters in the nursery and grow-finish phase, another

trial in a commercial setting following all litters to market would still be useful, as no other research has looked at this before.

7.11 Overall conclusions

Overall, the results of this research support the hypothesis that low litter birth weight is due to negative effects of intrauterine crowding in early gestation on placental development, which affects fetal development, and preprograms the litters to have poorer postnatal growth performance. However, more research is needed in several areas to link prenatal events to postnatal outcomes. Nonetheless, it is clear that the swine industry should strive to decrease the number of LBW litters. Until this has been established, management strategies to deal with LBW litters should be put in place.

Although maternal marine-oil based n-3 LCPUFA supplementation during (parts of) gestation and lactation shows some benefits, the results of this research also found some negative effects. Overall, therefore, supplementing gilt and sow diets with marine-oil based n-3 LCPUFA does not seem economically beneficial.

Table 7-1. Comparison of the characteristics of birth litters between 9 and 16 total piglets born, and classified as low (LBW), medium (MBW), high (HBW), or medium/high (MHBW) birth weight, using the data from the trials described in Chapters 4 and 5. Where parameters differed between studies, the P-values are bolded

	Chapter 4			Chapter 5		P-values	
	LBW	MBW	HBW	LBW	MHBW	Ch.4	Ch.5
Litter data							
n	42	82	24	25	72		
TB	12.7	12.9	13.5	12.9	12.9	0.30	0.96
BA	11.3	11.7	12.5	11.5	12.1	0.14	0.28
BA (% of TB)	89.5	91.2	93.2	88.8	93.9	0.47	**0.06**
SB	1.3	1.2	0.9	1.4	0.8	0.55	**0.07**
SB (% of TB)	10.5	8.8	6.8	11.2	6.3	0.38	**0.07**
Mummies	0.4	0.4	0.1	0.7	0.2	0.35	**<0.01**
Litter ave bw (kg) [A]	1.12 [a]	1.45 [b]	1.79 [c]	1.11	1.50	<0.001	<0.001
Total litter bw (kg) [A]	14.12 [a]	18.58 [b]	23.68 [c]	14.23	19.10	<0.001	<0.001
Ave placental wt (kg) [B]	0.21 [a] (n=16)	0.26 [b] (n=48)	0.28 [b] (n=10)	0.22 (n=9)	0.28 (n=41)	0.01	0.01
Necropsied pig data							
n	25	51	13	31	57		
Individual bw (kg)	1.03 [a]	1.41 [b]	1.84 [c]	0.91	1.26	<0.001	<0.001
Brain wt (g)	28.74 [a]	29.48 [a]	31.42 [b]	27.03	27.65	0.01	**0.41**
Liver wt (g)	36.82 [a]	48.02 [b]	56.53 [c]	30.48	40.26	<0.001	<0.01
Small intest. wt (g)	34.38 [a]	49.72 [b]	56.60 [b]	32.51	44.82	<0.001	<0.01
Muscle wt (g)	1.90 [a]	2.39 [b]	3.02 [c]	1.46	2.36	<0.001	<0.001
Brain:liver wt ratio	0.83 [a]	0.66 [b]	0.63 [b]	0.94	0.78	0.001	<0.05
Brain:intest. wt ratio	0.88 [a]	0.63 [b]	0.57 [b]	0.98	0.70	<0.001	0.001
Brain:muscle wt ratio	16.24 [a]	13.50 [b]	11.38 [b]	21.27	13.05	<0.01	<0.001

TB=total born, BA=born alive, SB=stillborn, ave=average, bw=birth weight, wt=weight, intest.=intestine

[A] Total number of pigs born in litter used as covariate

[B] Only taking into account litters where more than 50% of the placentae were recovered

[a, b, c] LSMeans in a row within a Chapter with different superscripts are significantly different at P<0.05

Table 7-2. Comparison of carcass characteristics between studies. In Chapter 4, litters were divided in low (LBW), medium (MBW) and high (HBW) birth weight. In Chapter 6, the litters with the lowest birth weights were chosen, so that most litters fell in the LBW category, and a few fell in the lower end of the MBW category. Only information of litters from sows fed control diets in Chapter 6 are shown.

	Chapter 4			Chapter 6	
Carcass data	LBW	MBW	HBW	Barn A	Barn B
n	9	15	5	44	174
Age at slaughter (days)	174.7 [a]	170.9 [b]	165.6 [c]	173.3	173.3
Live weight (kg)	115.8	116.0	116.5	126.9	123.0
Hot carcass weight (kg)	88.2	88.4	88.0	94.6	94.3
Loin depth (mm)	71.05	71.61	70.22	69.86	70.68
Fat depth (mm)	15.38	15.61	14.55	18.23	17.94
Lean meat (%)	56.4	56.4	56.5	55.4	55.8
Yield (%)	76.1	76.3	75.6	74.5	75.1
Grade premium (US$)	6.15	6.16	6.22	6.23	6.67
Sort loss (US$)	-0.93	-0.83	-0.80	-1.06	-1.70

[a, b, c] LSMeans in a row within a Chapter with different superscripts are significantly different at $P<0.001$

Table 7-3. Estimated economic impact (in Canadian dollars) of individual criteria when comparing litters with different litter average birth weight (modeling performed by Ken Engele, Prairie Swine Centre). Data of the study described in Chapter 4 was used for this analysis

Litter size comparison	BA	PWM	BA + PWM	Nurs. + GF	Total/pig	Whole herd basis
LBW vs. HBW	$7.85	$8.74	$16.64	$1.55	$18.09	$2.89
MBW vs. HBW	$5.06	$3.64	$8.46	$0.99	$9.45	$6.14
LBW vs. MBW	$2.80	$5.10	$8.08	$0.56	$8.64	$1.38

BA=born alive, PWM=pre-weaning mortality, nurs. + GF= nursery and grow-finish performance, LBW=low birth weight litters, MBW=medium birth weight litters, HBW=high birth weight litters

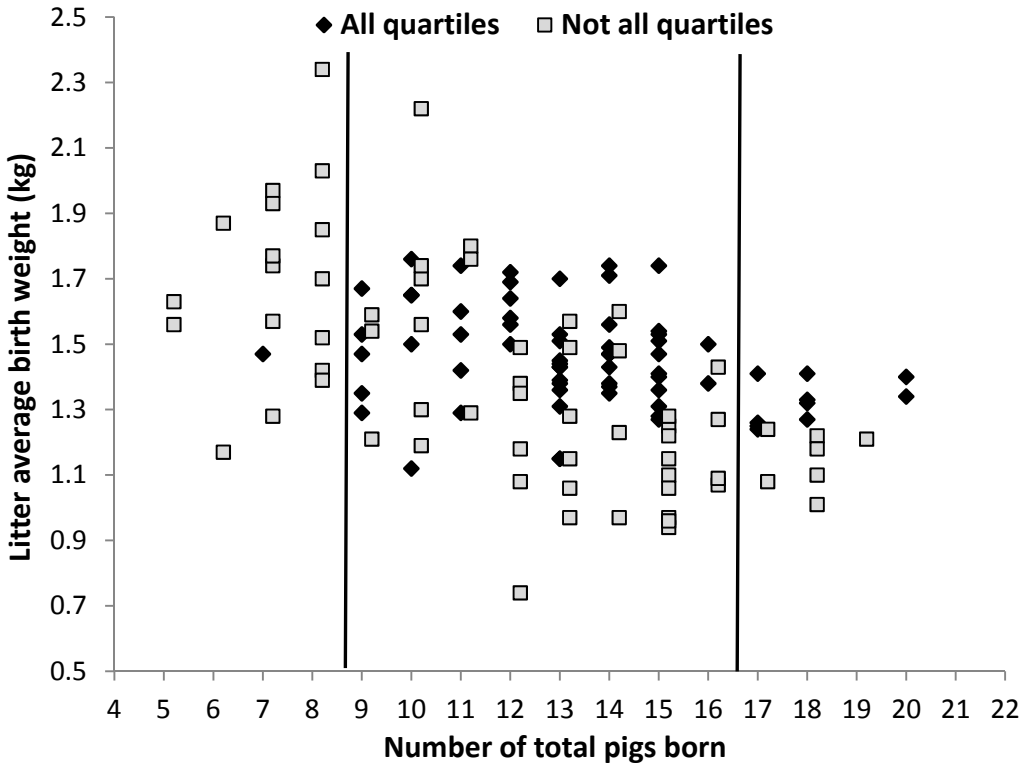

Figure 7-1. Relationship between litter size and litter average birth weight for litters from the trial described in Chapter 5, divided into litters that had piglets with individual birth weight in four different quartiles (0.80-1.25 kg, 1.25-1.50 kg, 1.50-1.75 kg and 1.75-2.50 kg; All quartiles, n=68) and litters that did not have piglets with individual birth weight in all four quartiles (Not all quartiles, n=66). Only piglets that were alive at the time of measuring birth weight were taken into account in this analysis. The litters between the two vertical lines are the litters with 9 to 16 total pigs born, which were classified as low, medium or high birth weight (see Chapter 5)

7.11 References

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