The peripartal sow:

A challenge for nutrition

An Cools

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Promotors:

Prof.dr.ir. Geert Janssens

&

Prof.dr. Dominiek Maes

Laboratory of Animal Nutrition, Department of Nutrition, Genetics, and Ethology

&

Department of Obstetrics, Reproduction, and Herd Health

Faculty of Veterinary Medicine

Ghent University

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An Cools

Vakgroep Voeding, Genetica en Ethologie,

Vakgroep Verloskunde, Voortplanting en Bedrijfsdiergeneeskunde

Faculteit Diergeneeskunde

Universiteit Gent

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List of abbreviations

General abbreviations

AA	Arachidonic Acid
ADF	Acid Detergent Fibre
Adj R^2	Adjusted R Square
ADL	Acid Detergent Lignin
AFD	Apparent Fecal Digestibility
AIA	Acid Insoluble Ash
ALA	α-Linolenic Acid
ANOVA	Analyses of Variance
ARC	Arcuate Nucleus
AUC	Area Under the Curve
BF	Back Fat
BW	Body Weight
CC	Correlation Coefficient
CREA	Creatinine
CTX	Serum CrossLaps
DHA	Docosahexaenoic Acid
DHGLA	Dihomo γ-Linolenic Acid
DPA	Docosapentaenoic Acid
ELISA	Enzyme Linked Immuno Sorbent Assay
EPA	Eicosapentaenoic Acid
ETA	Eicosatetraenoic Acid
FAME	Fatty Acid Methyl Esters
FFAR2	Orphan G-coupled Free Fatty Acid Receptor 2
FFAR3	Orphan G-coupled Free Fatty Acid Receptor 3
FRAP	Ferric Reducing Ability of Plasma
FSH	Follicle-Stimulating Hormone
GLA	γ-Linolenic Acid
GLP-1	Glucagon Like Peptide 1
GLP-2	Glucagon Like Peptide 2
GnRH	Gonadotropine-Releasing Hormone
GPx	Glutathione peroxidase
IVGTT	Intravenous Glucose Tolerance Test

LA	Linoleic Acid
LH	Luteinizing Hormone
LSD	Least Significant Differences
LW	Litter Weight
MDA	Malondialdehyde
ME	Metabolizable Energy
MMA	Metritis-Mastitis-Agalactia
MUFA	Mono-Unsaturated Fatty Acids
NDF	Neutral Detergent Fibre
NE	Net Energy
NEFA	Non-Esterified Fatty Acids
NFE	Nitrogen Free Extract
NPY	Neuropeptide Tyrosine
NSP	Non-Starch Polysaccharides
OC	Osteocalcin
OGTT	Oral Glucose Tolerance Test
PG	Prostaglandins
$PGF_{2\alpha}$	Prostaglandin $F_{2\alpha}$
PHS	Peripartal Hypogalactic Syndrome
PRRS	Porcine Reproductive and Respiratory Syndrome
PUFA	Poly-Unsaturated Fatty Acids
PYY	Peptide-Tyrosine-Tyrosine
RIA	Radio Immuno Assay
SCFA	Short Chain Fatty Acids
SD	Standard Deviation
SEM	Standard Error of the Means
SFA	Saturated Fatty Acids
SOD	Superoxide Dismutase
T_3	3, 3′, 5-Triiodothyronine
T_4	Thyroxine
TBARS	Thiobarbituric Acid Reactive Substances
TG	Triglycerides
VFI	Voluntary Feed Intake

Chapter specific abbreviations

Chapter 3

F0	Parturition feed 0 % fish oil
F1	Parturition feed 1 % fish oil
F2	Parturition feed 2 % fish oil
F3	Parturition feed 3 % fish oil
F4	Parturition feed 4 % fish oil
H_{50}	Point of 50 % hemolysis of erythrocytes
$H_{50}OF$	Sodium chloride concentration causing 50 % haemolysis
$H_{50}OS$	H_2O_2 concentration causing 50 % haemolysis
OF	Osmotic Fragility
OS	Oxidative Stability

Chapter 4

ADLIB	*Ad libitum* fed sows
C	Body condition
F	Feeding strategy
FAT	Sows with more than 22 mm back fat
LEAN	Sows with less than 18 mm back fat
MODERATE	Sows with back fat between 18 and 22 mm
RESTRICT	Restricted fed sows

Chapter 5

D	% nutrient in the feed
D_{AIA}	% acid insoluble ash in the feed
DMG	N,N-dimethylglycine
F	% nutrient in the feces
F_{AIA}	% acid insoluble ash in the feces

Chapter 6

ADLIB	*Ad libitum* fed sows
ADLIB-F	*Ad libitum* fed sows with more than 22 mm back fat
ADLIB-L	*Ad libitum* fed sows with less than 18 mm back fat
ADLIB-M	*Ad libitum* fed sows with back fat between 18 and 22 mm
C	Body condition
F	Feeding strategy
FAT	Sows with more than 22 mm back fat
LEAN	Sows with less than 18 mm back fat
MODERATE	Sows with back fat between 18 and 22 mm
STANDARD	Sows fed according to standard practice
STANDARD-F	Standard fed sows with more than 22 mm back fat
STANDARD-L	Standard fed sows with less than 18 mm back fat
STANDARD-M	Standard fed sows with back fat between 18 and 22 mm

Chapter 7

3OHC4	3-Hydroxybutyryl-carnitine
3OHC5	3-Hydroxyisovaleryl-carnitine + 2-Methyl-3-Hydroxybutyryl-carnitine
C2	Acetyl-carnitine
C3	Propionyl-carnitine
C3DC	Malonyl-carnitine
C4	Butyryl-carnitine + Isobutyryl-carnitine
C4DC	Methylmalonyl-carnitine
C5	Isovaleryl-carnitine + 2-Methylbutyryl-carnitine
C5:1	3-Methylcrotonyl-carnitine
C5DC	Glutaryl-carnitine
C6DC	3-Hydroxy-3-Methylglutaryl-carnitine
RS	Parturition feed with raw potato starch
SBP	Parturition feed with sugar beet pulp
WB	Parturition feed with wheat bran

Chapter 1

Introduction

1 Introduction: Peripartal feeding strategies of sows

1.1 The peripartal period: Lots of changes

The word "peripartal" is constructed form the Greek prefix "peri-" which can be translated as "around" and the scientific term "parturition" meaning "giving birth" (Lawrence, 2000). Although the term peripartal period is used by several authors, a clear definition of the time period it covers is usually not mentioned. In general, it relates to the period starting seven to five days prior to parturition, which coincides with the transfer from the gestation to the farrowing unit of the sow (Papadopoulos et al., 2009b), and ends several days to one week postpartum. During this period, sows have to cope with profound changes. The transition from gestating to lactating status, resulting in several physiological changes (described in 1.2), is the most obvious one. However, also more management related factors, such as housing and nutrition, are altered during the same short period of time which could interfere with sow's peripartal physiology.

1.1.1 Housing

As already mentioned the peripartal period starts with the transfer of sows from the gestation to the farrowing unit. First, the handling of sows during transfer can cause stress (Andersen et al., 2006). Apart from that, the shift from group housing, as legally required for gestating sows in the European Union (European guidelines for pig husbandry 2008/120/EC), to individual housing in the farrowing house, is a major change (Boyle et al., 2002). This is particularly stressful when sows are restrained in a farrowing crate (Jarvis et al., 2001; Oliviero et al., 2008), which are used to prevent excessive piglet losses due to crushing (Weary et al., 1996a). As crated sows commonly have no access to nesting material and have limited space allowance, they cannot express their nesting behaviour. The provision of nesting material such as sawdust (Cronin et al., 1993) or straw (Herskin et al., 1998) can improve maternal behaviour and reduce stress. Feeding fibrous diets throughout gestation can reduce activity of sows during the peripartal period (Farmer et al., 1995). However, this is probably due to increased satiety of the bulky gestation diet (Danielsen and Vestergaard, 2001) and will not affect nesting behaviour.

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As parturition is a critical event determining sows productivity, problems such as dystocia (Canario et al., 2006) or piglet crushing (Andersen et al., 2005) can drastically reduce number of weaned piglets. Therefore, frequent supervision during the peripartal period is recommended to prevent excessive losses (Holyoake et al., 1995). However, supervision should be done properly, else it can lead to stress, more stillborn piglets (Vanderhaeghe et al., 2010b) or increased crushing incidences (Weary et al., 1996a).

Along with the changes in the housing system, some environmental factors also change e.g. higher ambient temperature in the farrowing unit compared to the gestation unit. Especially when sows start farrowing, heating lamps and heated piglet nests can result in room temperature above the thermoneutral zone of the sow (18 - 22 °C). Whereas sows that suffer from heat stress during late gestation have more stillborn piglets (Omtvedt et al., 1971; Vanderhaeghe et al., 2010b), heat stress during early lactation will mainly result in depressed feed intake (McGlone et al., 1988; Messias de Bragança and Prunier, 1999; Renaudeau and Noblet, 2001). Quiniou and Noblet (1999) observed a quadratic decrease of voluntary feed intake during lactation with increasing room temperature. Especially, when environmental temperature rises above 25 °C feed intake decreased tremendously. This reduction of lactational feed intake will lead to decreased milk yield resulting in impaired litter performance (McGlone et al., 1988; Messias de Bragança and Prunier, 1999; Renaudeau and Noblet, 2001). When piglets have access to creep feed, they compensate the lowered milk output with increased creep feed consumption (Renaudeau and Noblet, 2001). Unfortunately, litter growth rate still remains lower than that of sows housed under thermoneutral conditions. Besides reduced milk production, some authors also reported altered milk composition. Results, however, are ambiguous: Renaudeau and Noblet (2001) reported an increased dry matter, ash, and energy content when temperature rose from 20 °C to 29 °C whereas the same authors in another study reported decreased fat, lactose, and calcium content comparing milk from sows kept at 20 °C versus sows kept at 28 °C (Renaudeau et al., 2003). The effect of environmental temperature on colostrum composition is not clear. One report (Christon et al., 1999) indicated decreased energy content of colostrum under tropical conditions in comparison to temperate environment. Surprisingly, these authors also reported an altered fatty acid profile of both colostrum and milk with lower levels of n-3

polyunsaturated fatty acids (PUFA) under tropical conditions. The authors did not report an explanation for the altered fatty acid profile of the milk but a possible hypothesis is that high ambient temperature resulted in increased capillary permeability in the mammary gland so lipolytic enzymes enter the milk. This may result in altered milk fat profiles like this is previously hypothesized in sheep by Sevi and Caroprese (2012). But more likely, the lower feed intake of the sows under tropical conditions limited the amount of PUFA available for secretion into the milk, resulting in an altered fatty acid profile of the milk. Possibly, there is also an effect of the peripartal diet on colostrum and milk composition and, therefore, more research on this topic is warranted.

1.1.2 Nutrition

It is well established that applying a two-diet feeding regime instead of one single diet for gestating and lactating sows improves productivity (Neil et al., 1996; O'Dowd et al., 1997). However, using a separate gestation and lactation diet implies that sows have to switch diets during the peripartal period.

Although a lot of research on the ideal composition of gestation and lactation diets is performed over the years and a wide variety of diet formulas exist, some general differences between both diets can be noted.

Energy demand of gestating sows increases significantly towards parturition, mainly by increased fetal growth and mammary gland developement (Close and Cole, 1986). Also throughout lactation, energy demand rises tremendously due to increasing milk production (Theil et al., 2004). Whereas daily energy intake for gestating sows is estimated between 20 and 35 MJ NE/day (Close and Cole, 1986; Noblet et al., 1997), energy demand for lactating sows exceeds 50 MJ NE/day (Theil et al., 2004), using the conversion equation $NE = 0.78 \times ME - 1.96$ (Just, 1982). In theory, increased energy demand during lactation could be covered by increased feed intake (National Research Council, 1998). However, voluntary feed intake throughout lactation is often limited due to physical (Forbes, 2009), metabolic (Black et al., 2009), and hormonal regulation (Carroll and Allee, 2009), and could be reduced further by stress and environmental factors (described in 1.1.1). In order to avoid excessive mobilization of body reserves and improve litter performance, a lactation diet is in practice mostly higher in energy in comparision to a gestation diet (Coffey et al., 1994). Parallel with increasing energy demand with the progression of the reproductive cycle,

starting from insemination onwards, also protein demand rises. Especially, due to the high need of proteins for milk production, it is recommended to increase the amount of protein present in a lactation diet in comparison to a gestation diet (National Research Council, 1998). Additionally, not only the protein content, but also the ideal amino acid profile differs between gestating and lactating sows (Kim et al., 2009). As excessive body condition gain towards parturition can cause problems with feed intake during lactation (reviewed by Eissen et al., 2000), sows are mostly fed restricted throughout gestation to prevent excessive gain of body condition (Weldon et al., 1994a). In order to improve sow's welfare and prevent stereotypic behaviour, gestation diets are often diluted by adding fibre (Danielsen and Vestergaard, 2001). Also, gestation diets rich in fibres improve feed intake throughout lactation (Farmer et al., 1996; Quesnel et al., 2009). The best results are achieved with a fibre rich gestation diet followed by a starch rich lactation diet (van der Peet-Schwering et al., 2003a). Hence, this implies a major difference in fibre content between gestation and lactation diet (Oliviero et al., 2009).

Besides the differences in nutrient content, the gestation and the lactation diet are also composed of different ingredients. This means that sows have to adapt to the altered taste of the diet. Moreover, the gastrointestinal tract of the sow has to adapt to the new dietary composition.

Concurrent with the transition from a gestation to a lactation diet, mostly also the feeding scheme is changed. Whereas gestating sows are mostly fed restricted to aim for optimal body condition at parturition (Young et al., 2004), lactating sows are often fed *ad libitum* to maximize feed intake and prevent excessive loss of body reserves (Koketsu et al., 1996).

1.2 Peripartal physiology: From gestation to lactation

1.2.1 Hormonal changes

At the end of gestation the uterus becomes too small for the fetuses. As a result, fetuses get stressed and their adrenal cortex starts producing cortisol (Bazer and First, 1983; Davidson and Stabenfeldt, 2007). This rise in fetal cortisol initiates the parturition process by affecting steroid synthesis in the sow. Furthermore, there is a shift from progesterone towards estrogen synthesis (Davidson and Stabenfeldt, 2007). These elevated estrogen levels induce the production of prostaglandins (PG) by the myometrium (Abayasekara and Wathes, 1999; Bazer

and Thatcher, 1977), leading to luteolyses and decreased progesterone concentrations in sows' blood (Baldwin and Stabenfeldt, 1975; Bazer and First, 1983; Ellendorff et al., 1979). This drop in progesteron starts approximately two days prepartum (Bazer and First, 1983; Ellendorff et al., 1979; Foisnet et al., 2010b) and as a result, inhibition of myometrium contractions fades out and cervical dilatation starts (Davidson and Stabenfeldt, 2007). Furthermore, prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) is probably the most important hormone in the entire parturition cascade. Besides luteolysis, which results in decrease of progesteron and a release of relaxin (Davidson and Stabenfeldt, 2007), it also stimulates the pituitary gland to synthetize both oxytocin (Ellendorff et al., 1979) and prolactin (de Passille et al., 1993). Whereas relaxin is responsible for softening of the cervix (Cowart, 2007) and relaxation of the pelvic ligaments and muscles (Davidson and Stabenfeldt, 2007), oxytocin is responsible for myometrial contractility that leads to expulsion of the fetuses (Cowart, 2007). A visualisation of the interactions of the different hormones is shown in Figure 1.1.

Prolactin, also secreted by the posterior pituitary gland, is involved in milk secretion in the mammary gland whereas oxytocin is important for milk removal of the udder (Davidson and Stabenfeldt, 2007). Both hormones are released as a result of a suckling induced reflex.
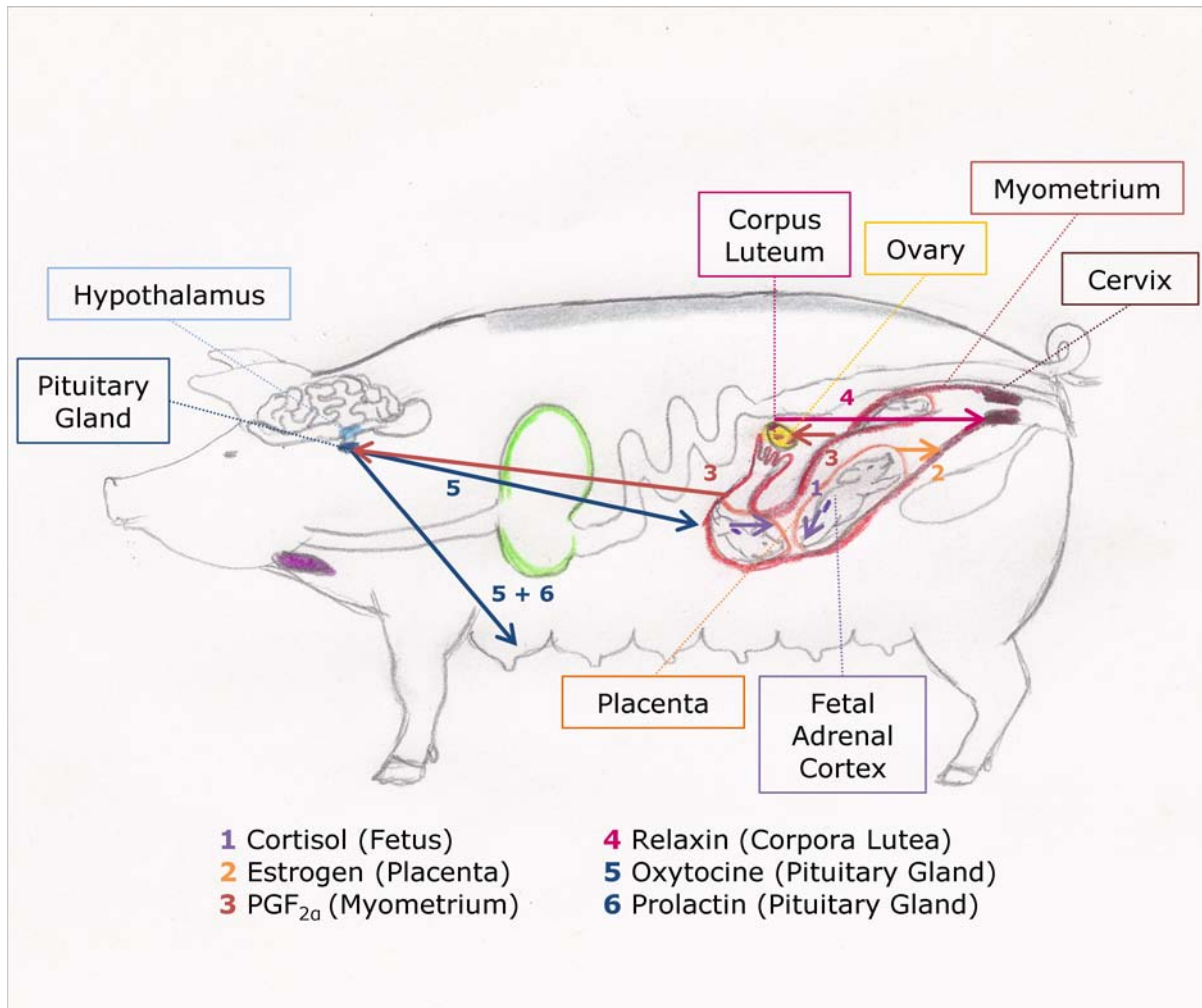
Figure 1.1 Overview of the hormonal cascade initiating parturition. Arrows indicate the origine and target of the different hormones, color of the arrows links to the secreting tissue. The cascade starts with the secretion of cortisol by the fetal adrenal cortex (1) which finally results in contractions of the uterus and secretion of colostrum (own picture).

In general, these hormonal changes are similar for each sow. However, external factors could affect these hormones and interfere with the parturition process and the subsequent lactogenesis. As already mentioned, alteration of housing and feeding management can cause stress resulting in increased cortisol levels (Oliviero et al., 2008). The elevated cortisol can slow down the parturition progress (Lawrence et al., 1995), affect maternal behaviour of sows (Mainau and Manteca, 2011) and reduce milk excretion (Algers and Uvnas-Moberg, 2007) probably via affected oxytocine functioning (Lawrence et al., 1995). Additionally, suboptimal feeding resulting in a disturbed energy balance at the time of parturition also affects these reproductive hormones. Some appetite regulating hormones, such as leptin and ghrelin, are linked with energy homeostasis and reproduction (Budak et al., 2006). Leptin is mainly secreted by the adipose tissue

(Barb et al., 2005) and is anorexigenic (Barb et al., 2001). Ghrelin is mainly produced in the stomach mucosa (Govoni et al., 2005) and is orexigenic (Budak et al., 2006).
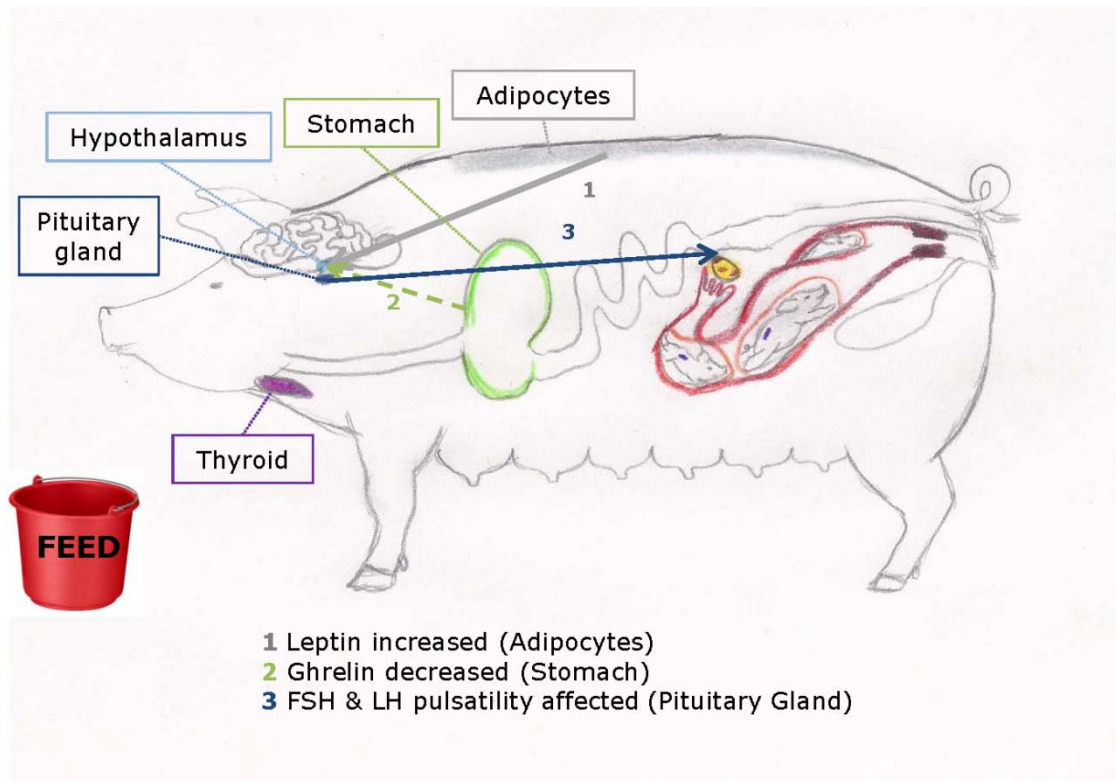

Figure 1.2 When sows are fed, their leptin levels rise and secretion of ghrelin decreases. One of the consequences is that the pulsatility of LH and FSH changes (own picture).

Both hormones act on the arcuate nucleus (ARC) of the hypothalamus where they affect the expression of neuropeptide Y (NPY) and modulate the pulsatility of the gonadotropin-releasing hormone (GnRH) (Barb et al., 2001; Dong et al., 2009), consequently affecting follicle-stimulating hormone (FSH) and luteinizing hormone (LH) production (Greco and Stabenfeldt, 2007) (Figure 1.2). It is, therefore, obvious that altering the feeding strategy of peripartal sows may affect hormones like leptin and ghrelin, and consequently affect reproduction hormones.

1.2.2 Metabolic changes

With parturition and the onset of lactation not only a lot of hormones change, but also the metabolism of sows is altered. As a result of increased nutrient demands for final fetal development and lactogenesis, sows become catabolic and start using their body reserves (Close et al., 1985; Le Cozler et al., 1999). In sows the three most important tissues that can compensate for shortage of nutrient intake are fat tissue, muscle or lean tissue, and bone tissue. For each of these tissues

several metabolites or markers could be selected to monitor the metabolism of a specific tissue. These markers are very useful to closely evaluate the impact of a peripartal feed or feeding scheme on the metabolism of the sow.

Shortage of energy is mostly compensated by catabolism of fat reserves. This catabolic state is reflected in increased concentration of non-esterified fatty acids (NEFA) in the plasma with a peak value on the day of parturition (Le Cozler et al., 1999). Part of the peak could possibly be explained by the high amounts of energy needed for the physical exercise the parturition actually is. The energy metabolism during lactation depends on the sow's body condition at the end of gestation (Hulten et al., 1993; Revell et al., 1998b) and can also be influenced by the energy intake of the sow (Revell et al., 1998b; Weldon et al., 1994a). The alteration of the fat metabolism is in most cases accompanied by changes in the protein metabolism. This is not surprising as low energy intake mostly implies a reduced protein intake. Catabolism of lean tissue is often measured by means of increased creatinine levels (Hulten et al., 2002a; Yang et al., 2000). Not only reduction of total feed intake during the peripartal period (Neil, 1996), but also reduced protein intake (Reese *et al.*, 1984), or lowered lysine intake (Yang et al., 2000) can alter creatinine levels.

A third important reserve tissue is the bone. It is known that the skeleton of the sow is the major source of calcium during both gestation and lactation (Giesemann et al., 1998). It can be expected that bone metabolism is high during the peripartal period because of high calcium demand for uterus contractions during farrowing as well as for the onset of lactation (Mahan and Vallet, 1997). As serum calcium levels are rather stable in sows throughout the reproduction cycle (Giesemann et al., 1998; Liesegang et al., 2005; Mahan and Vallet, 1997), bone markers can be a useful alternative to monitor the bone metabolism of sows (Liesegang et al., 2005). Osteocalcin (OC) as a marker for bone formation (Lauridsen et al., 2010; Liesegang et al., 2002) and serum crosslaps (CTX) as marker for bone resorption (Christgau et al., 1998; Liesegang et al., 2005; Rosenquist et al., 1998) can provide more insight into the bone turnover of peripartal sows and indirectly provide some insight into the calcium metabolism during this period.

1.2.3 Metabolic disorders

With parturition and the onset of lactation the metabolic demand of sows increases tremendously (Le Cozler et al., 1999; Mosnier et al., 2010). For dairy cattle, it is well described that due to the physiological challenges during the transition period, the dairy cow is susceptible to several metabolic disorders like hepatic lipidosis (Grummer, 1993), ketosis (Guo et al., 2008), or parturient paresis (Goff and Horst, 1997). They mostly result from an increased need for energy (Ingvartsen, 2006; van Dorland et al., 2009) and nutrients (Goff and Horst, 1997) with the onset of lactation. Given the increased production results of modern sows, it is likely that peripartal sows are also facing considerable metabolic challenges. Ketosis in peripartal sows is rare (Alsop et al., 1994; Theil et al., 2012), but periparturient hypogalactic syndrome (PHS), resulting in impaired lactational performance (Hansen et al., 2012), is commonly diagnosed in modern sow herds (Papadopoulos et al., 2010; Preissler et al., 2012). It is questionable whether PHS in sows is preceded by metabolic disorders similar to those described for dairy cattle. Although the exact cause of PHS is not yet clear, potential risk factors related to the occurrence of PHS were described. High feed intake (Papadopoulos et al., 2010) and obese body condition (Göransson, 1989b), both at the moment of farrowing are identified as predisposing factors. Similar risk factors (over conditioning at calving (Goff and Horst, 1997; Hayirli et al., 2002), overfeeding during the dry period (Dann et al., 2005; Ingvartsen, 2006)) are described for peripartal metabolic disorders in dairy cattle. Despite these similarities, in sows little is known about the profile of relevant metabolic parameters during the peripartal period in relation to those risk factors. Also, information considering proper transition or peripartal feeding strategies (diet composition and feeding schemes) is scarce.

1.3 Peripartal feeding strategies

It is well established that sow performance is improved when offering sows a separate gestation and lactation diet (Neil et al., 1996; Neil and Ogle, 1996; O'Dowd et al., 1997). Furthermore, it is also clear that around parturition the metabolism of the sow changes tremendously and the sow has to deal with several metabolic changes (described in 1.2). It could, therefore, be assumed that during this particular period the highly prolific sow has specific dietary needs. Whereas for dairy cattle the importance of a specific transition diet is well

described (Guo et al., 2007), information about peripartal feeding for sows is much more scarce. Although in practice transition or parturition diets for sows are used, scientific literature concerning diet composition is scarce.

The main metabolic challenge for the peripartal sow is to prevent excessive catabolism, which often occurs some days before parturition (Close et al., 1985). To prevent excessive catabolism the nutrient composition of the diet can be changed or the feeding level can be adjusted. Besides excessive catabolism, some more specific problems such as early parturition, reduced piglet vitality, hypophagia, or hypogalactia, affect sow's productivity. Although the main goal of dietary ingredients is to deliver energy, protein, fibres, vitamines, or minerals, some particular ingredients can also contribute to the sows' metabolism in a more specific manner.

1.3.1 Peripartal diet composition: Functional ingredients

1.3.1.1 Energy source: Fat or carbohydrates?

It is well established that lack of energy during late gestation lowers piglet birth weight (Coffey et al., 1994) whereas extra energy throughout lactation increases litter weight gain (Coffey et al., 1994) and prevents excessive mobilization of sows' body reserves (Theil et al., 2004). As a source of energy, both fat and carbohydrates can be used. Although both energy sources are efficiently used by the sow, research has pointed out that they do not only deliver energy, but also affect the metabolism. Supplementing sow diets during late gestation and throughout lactation with additional fat resulted in increased preweaning piglet survival (Quiniou et al., 2008; Seerley et al., 1974), increased milk and milk fat production (Averette et al., 1999; Coffey et al., 1982; Coffey et al., 1987). Hence, the increased milk fat output of these sows resulted in fatter piglets at weaning (Seerley et al., 1974; Tilton et al., 1999; van den Brand et al., 2000), and in more mobilization of body fat reserves (Quiniou et al., 2008). In general, sows offered extra energy out of fat also became more catabolic (van den Brand et al., 2000). Additionally, fat fed sows were more glucose intolerant in comparison to sows that received extra starch during late gestation and lactation (van der Peet-Schwering et al., 2004). This reduced glucose tolerance of sows during late gestation has been related with increased preweaning piglet mortality (Kemp et al., 1996). Furthermore, Newcomb et al. (1991) reported higher fasting glucose levels of piglets when their dams were fed soybean oil in

comparison to sows fed starch as main energy source. As soybean oil is rich in linoleic acid (LA 18:2n-6), which is an n-6 polyunsaturated fatty acid (PUFA), this result is not surprising. Consumption of high amounts of n-6 PUFA was previously associated with the occurrence of insulin resistance (Storlien et al., 1997) which could explain the elevated fasting glucose levels of the offspring of soybean oil fed sows (Newcomb et al., 1991) and the reduced glucose tolerance of the sows themself (van der Peet-Schwering et al., 2004). Similarly, also increased amounts of corn oil (Seerley et al., 1974) or sunflower oil (Papadopoulos et al., 2009b), both rich in n-6 PUFA, fed to sows during late gestation resulted in increased fasting glucose of the piglets or increased insulin levels of the sows, respectively. On the contrary, offering a diet high in n-3 PUFA during late gestation resulted in lowered insulin and leptin levels of the sows (Papadopoulos et al., 2009b). This confirms that increased consumption of n-3 PUFA improves insulin sensitivity (Storlien et al., 1997). Moreover, *in utero* exposure of piglets to n-3 PUFA improved the piglets' intestinal glucose absorption and muscle glycogen reserves (Gabler et al., 2007). This could be explained by the increased expression of both glucose transporter 2 and sodium glucose transporter 1 protein in the jejunum of piglets from n-3 PUFA fed sows (Gabler et al., 2007) Besides this effect on glucose metabolism, it is also known that n-3 PUFA, and mainly docosahexaenoic acid (DHA, 22:6n-3), are important for fetal development (Innis, 2005; Innis, 2007). Therefore, supplementation of sow's gestation and lactation diet with different types of oils should be done carefully, especially, when considering the importance of the ratio of n-6/n-3 PUFA for fetal development and sow's metabolism.

1.3.1.2 Protein intake: Specific amino acids do matter

It is not surprising that highly prolific sows need sufficient amounts of protein for fetal development during gestation and for milk production throughout lactation. Limited protein supply to gestating sows resulted in sows with higher back fat levels and reduced lactational feed intake (Mahan, 1998) on the one hand, and impaired fetal development on the other hand (Kim et al., 2009). This protein restriction of gestating sows leads to impaired myofiber formation of the offspring implying lower piglet birth weight and fatter slaughter pigs (Rehfeldt et al., 2012). On the contrary, excessive protein intake during gestation had no effect on fetal developement (Rehfeldt et al., 2012), mammary gland

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development (Kusina et al., 1999; Weldon et al., 1991), or colostrum composition (Al-Matubsi et al., 1998). Hence, it is important to supply gestating sows with the necessary amount of dietary protein to prevent production losses, but excessive intake should be prevented as well as this is both cost inefficient and has a negative impact on the environment. Therefore, more research on phase feeding of gestating sows is warranted. Moreover, also the specific amino acid profile of sow feed deserves more attention. Although basically amino acids are protein building blockes, they are also identified as key regulators in the sow's metabolism. Arginine for instance, was identified as detrimental for mammary gland vascularisation via production of nitric oxid (Kim and Wu, 2009; O'Quinn et al., 2002). Furthermore, it is well established that lysine is the first limiting amino acid for sows: it is not only detrimental for milk production (Tokach et al., 1992), and mobilization of body protein reserves (Dourmad et al., 1998; Touchette et al., 1998), but suboptimal lysine intake throughout lactation has a negative effect on somatotropine hormones resulting in a reduced postweaning ovulation rate (Mejia-Guadarrama et al., 2002). Depending on whether lactating sows actually become catabolic or not, the order of the essential amino acids after lysine can change. When sows are catabolic and mobilize body reserves threonine has been identified as the second most important essential amino acid whereas, when sows are able to compensate milk production with sufficient feed intake, valine is the second most important essential amino acid (Kim et al., 2001). Moreover, research has pointed out that the uptake of branched chain amino acids (valine, leucine, and isoleucine) by the mammary gland largely exceeded output in the milk (Li et al., 2009b) and that increased consumption of these branched chain amino acids by lactating sows increased milk protein output (Dunshea et al., 2005). This is not surprising given that these branched chain amino acids are catabolized by the mammary gland (Nielsen et al., 2002) and used to produce glutamine and aspartate both needed for milk protein synthesis (Li et al., 2009b). In a similar way, also arginine is catabolized by the mammary gland to glutamine and proline, again in favor of milk protein production (O'Quinn et al., 2002). However, amino acid profiles should be modified carefully and unbalanced amino acid profiles could result in production losses instead of improved productivity. Laspiur et al. (2009) reported reduced piglet and sow performance when crude protein content of the lactation diet exceeded 18 %. However, as in this study diets were made isocaloric on

metabolizable energy and the energy efficiency is lower for crude protein than for other energy sources like fat or carbohydrates. The reduced performance of the high crude protein group could be caused by lack of net energy and not by an excess of crude protein. Also lowering the ratio of tryptophan to branched chain amino acids resulted in reduced voluntary feed intake of lactating sows (Trottier and Easter, 1995) which again impaired sows productivity and resulted in excessive body protein mobilization.

1.3.1.3 Fibres and fermentable substances: Feeding the intestinal microbiota

The advantages of fibre in the feed of gestating sows on health and behaviour are well known (Meunier-Salaun et al., 2001). Stereotypic (Bergeron et al., 2000; van der Peet-Schwering et al., 2003b) and agressive behaviour (Danielsen and Vestergaard, 2001) are lower when using bulky gestation diets. However, they may also influence the energy metabolism of the sow (Le Goff and Noblet, 2001; Noblet and Le Goff, 2001). Depending on the fibre type used, energy expenditure was reduced either by increased satiety and reduced activity (Souza da Silva et al., 2012), or by production of short chain fatty acids (SCFA) as a result of hindgut fermentation (Anguita et al., 2007; de Leeuw et al., 2008). In Figure 1.3 an overview of how and where different types of fibres act on the gastrointestinal tract.

	Soluble fibre: Oat bran Raw potato starch	Mixed fibres: Sugar beet pulp	Insoluble fibre: Wheat bran Straw
Properties	Viscous	Bulky	Bulky
Stomach	Gastric emptying ↘		
Small intestine	Transit time ↘		
Proximal colon	Fermentation	Transit time ↘ Fermentation	
Distal colon		Water binding	Transit time ↘

Figure 1.3 Overview of the different types of fibrous ingredients frequently used in sow diets and their effect of gastrointestinal tract (after James et al., 2003).

These SCFA were not only used as alternative energy sources (Noblet and Le Goff, 2001; Souza da Silva et al., 2012) but were also able to affect the metabolism of the sow probably via activation of the orphan G-protein-coupled free fatty acid receptor 2 and 3 (FFAR2 and FFAR3) (Haenen et al., 2013). Both FFAR2 and FFAR3 were activated by SCFA and were associated with increased production of peptide-tyrosine-tyrosine (PYY) by colonocytes, responsible for reduction of gastrointestinal motility (Cuche et al., 2000; Darzi et al., 2011). Furthermore, SCFA as a result of hindgut fermentation also resulted in increased secretion of both glucagon-like peptide 1 and 2 (GLP-1 and GLP-2) by the L-cells in the small intestine (Burrin et al., 2003). Similar to PYY, GLP-2 reduced gastrointestinal motility (Burrin et al., 2003) whereas GLP-1 increased insulin secretion and sensitivity (Freeland and Wolever, 2010; Tolhurst et al., 2012), and reduced gastric emptying (Freeland et al., 2010). Besides beneficial effects of fibre rich diets on the microbiota of the hindgut, also a carry-over effect towards lactational feed intake was reported (Farmer et al., 1996; Quesnel et al., 2009). The mechanism for this increased feed intake remains, however, unclear. Finally, fibre rich diets during the peripartal period also decrease the incidence of constipation (Oliviero et al., 2009; Tabeling et al., 2003) resulting in improved sow welfare (Mainau and Manteca, 2011) and less dystocia (Oliviero et al., 2010). Despite all this knowledge on the potential effects of fibres in sow diets,

the effects of different types of fibres during the peripartal period are not known. Also considering the effects of different types of fibres on the metabolism of the sow, much more research is warranted.

1.3.1.4 Digestibility enhancers: Upgrade the nutritional value of the diet

By enhancing the digestibility of sow feed, feed efficiency was improved and a broader range of ingredients became usable for feed formulation (Barletta, 2001). A frequently used enzyme in sow diets is phytase. Since the ban on the use of animal derived feedstuff such as meat and bone meal (European Regulation considering animal by-products 1069/2009/EC), phytase supplemenation became important to improve the phosphorus digestibility of wheat based diets. By adding phytase to the sow diet, phosphorus inclusion can be lowered without negative consequences for the sows health (Liesegang et al., 2005) and with beneficial effects on the environmental phosphorus burden (Eriksson et al., 2005). Considering other digestibility enhancing enzymes, most research is performed on nursery pigs. Supplementation of sow's diets with cellullase or xylanase to enhance digestibility of fibres (Diebold et al., 2004) would be of less interest given that sows are able to ferment these substances in their hindgut (described in 1.3.1.3). On the contrary, adding lipase or emulsifying agents to sow diets could be beneficial. Fat is often used in sow feed around parturition to augment energy content (Cieslak et al., 1983), and therefore, improvement of fat digestibility could be beneficial. Also as fat addition to a diet often decreased the digestibility of the other components by making the feed less accessible for digestive enzymes (Dierick and Decuypere, 2004; Le Goff and Noblet, 2001). Therefore, addition of products like lipase or emulsifiers were not only able to improve digestibility of the fat fraction of the diet but also improved the digestibility of the fat free fraction (Dierick and Decuypere, 2004). But despite this knowledge, almost all research on this topic is performed in nursery piglets and hardly any reports are dealing with producing sows.

1.3.2 Peripartal feeding schemes: How much sows should eat?

In practice, gestating sows are commonly fed restricted to prevent excessive gain of body condition whereas lactating sows are mostly fed *ad libitum* to minimize body condition losses as much as possible (Koketsu et al., 1996). However, sows already become catabolic during late gestation (Le Cozler et al., 1999). Therefore, they could benefit from increased feed intake during late

gestation. Surprisingly, in literature only a few studies previously investigated this type of feeding strategy for peripartal sows. When *ad libitum* feeding was introduced at or shortly postpartum several authors reported a problematic feed intake during early lactation (Anil et al., 2006; Koketsu et al., 1996). Furthermore, high feeding levels prepartum were identified as risk factors for some metabolic disorders such as hypogalactia (Göransson, 1989b) whereas Neil et al. (1996) reported increased incidence of hypogalactia when *ad libitum* feeding was introduced postpartum. In most cases, feed intake during early lactation was gradually increased to prevent total or partial anorexia of the lactating sows (Koketsu et al., 1996; Mosnier et al., 2010). To our knowledge, there is only one study investigating the effect of *ad libitum* feeding prepartum (Neil, 1996). This latter study did not report any negative effects on sow or piglet performance. On the contrary, when *ad libitum* feeding was introduced prepartum, sows were more capable of maintaining a proper body condition throughout lactation. Therefore, *ad libitum* feeding during the peripartal period could be a usefull solution to prevent sows becoming catabolic and further research on this topic is warranted.

1.4 Conclusion

It is clear that although many studies were done on gestation and lactation feed for sows, there is need for more clarity considering the actual feeding strategies applicable on peripartal sows. Although it is known that several functional ingredients like specific fatty acids, amino acids, or functional fibres are able to modulate the sow's metabolism, it is, however, not always clear whether using these metabolic modulating ingredients during the peripartal period could improve productivity and metabolism of peripartal sows. Also feeding strategies in the prevention of sows becoming catabolic at late gestation deserve further clarification. Whether it is better to improve diet digestibility or aiming for maximized feed intake warrants further research.

Chapter 2

Aims and objectives

2 Aims and objectives

Litter size of sows has increased dramatically over the last decades. Commercial sow herds are weaning over 28 piglets per sow per year. The metabolic demands for these highly prolific sows have changed concurrently, and optimal feeding at each moment of the reproduction cycle is, therefore, warranted. One of the critical moments in the sow's reproduction cycle is the peripartal period. During this rather short time span, sows have to cope with many changes such as different housing (transfer from gestation unit to farrowing room), changes in reproductive hormones, and also metabolic demand alters due to the shift from a gestating to lactating state. Although sows have specific needs during the peripartal period, little research on dietary requirements and management is performed within this topic.

The general aim of the present thesis is to investigate how targeted peripartal feeding could deal with frequently occurring problems, prevent sows from suffering from peripartal problems, and contribute to improvement of performance in highly prolific sows.

To prevent early parturition and improve piglet viability, fish oil can be used as a functional ingredient in sow diets. However, little is known about the optimal dose that should be used and also about eventual trade-offs in the use of fish oil not much has been published yet. Therefore, we want to test different doses of fish oil in parturition feed of sows and monitor eventual side effects (**Chapter 3**). Herewith, special attention will be paid to oxidative stability of erythrocyte membranes as n-3 polyunsaturated fatty acids are highly susceptible to oxidative stress.

A second reoccurring problem is peripartal hypophagia. Although peripartal hypophagia frequently occurs in modern sow herds, and several risk factors for this problem were identified, little is known about the physiological background of this problem. Given that high feed intake and high body condition both during late gestation are risk factors, a peripartal profile of feed intake regulating hormones will be constructed in order to get a more elaborated view on peripartal feed intake regulation (**Chapter 4**).

By the end of gestation highly prolific sows often become catabolic with detrimental consequences for the fetal development. To overcome the problem of energy shortage and to assure proper nutrient intake during the peripartal period

digestibility of the diet could be improved by adding an emulsifying agent to the diet. Therefore, the effect of N,N-dimethylglycine supplementation, with emulsifying properties and the potential to affect the glucose metabolism, in parturition diets will be tested to improve the energy supply of the peripartal sow (**Chapter 5**).

Besides improved digestibility, a second strategy in preventing excessive mobilization of body reserves as a result of increased metabolic demands could be to adjust the amount of feed offered. Therefore, the effect of *ad libitum* feeding in comparison with a commonly applied restricted feeding scheme will be investigated (**Chapter 6**).

Together with a catabolic state at late gestation and hypophagia at early lactation, modern sows often suffer from peripartal constipation. All these aspects result in reduced welfare of the sow and mostly reduce milk production, which is detrimental for piglet growth. To overcome the problems of constipation and reduced feed intake fibres could be added to the diet. It is, however, not clear which types of fibre are most optimal in the prevention of these latter mentioned problems. In a fifth study the effects of different functional fibres included in a parturition diet on peripartal constipation and lactational feed intake will be compared (**Chapter 7**).

Chapter 3

Dose-response effect of fish oil in parturition feed

Dose-response effect of fish oil substitution in parturition feed on erythrocyte membrane characteristics and sow performance

A. Cools, D. Maes, G. Papadopoulos, J.-A. Vandermeiren, E. Meyer, K. Demeyere, S. De Smet, G.P.J. Janssens

The present study aimed to investigate whether n-3 polyunsaturated fatty acids (PUFA) incorporate into erythrocyte membranes of peripartal sows in a dose-responsive manner and whether the altered fatty acid profile affects the cell membrane characteristics. At day 109 of gestation (day 0), 51 sows were divided into five treatment groups. Each group received a diet with a different ratio of fish oil to pork lard for nine consecutive days. Blood samples were taken at day 0 and 10 days later. The fatty acid profile of erythrocytes was determined, as well as the osmotic fragility and oxidative stability of erythrocytes. Thiobarbituric acid reactive substances (TBARS) and ferric reducing ability of plasma (FRAP) were determined in plasma samples. Finally, reproductive and performance parameters of both sows and piglets were recorded until weaning. Supplementation of fish oil during the peripartal period changed the fatty acid profile of erythrocyte membranes in a dose-responsive manner. Although the n-3 PUFA content of erythrocyte membranes increased with increasing amounts of fish oil in the diet, no significant effect on erythrocyte osmotic fragility could be recorded. In contrast, oxidative stability of erythrocytes decreased linearly with increasing amounts of fish oil in the diet. Similarly, both TBARS and FRAP linearly increased with increasing percentages of fish oil in the diet. Neither piglet nor sow performance was influenced by dietary treatments, except for a decrease of both piglet survival and weaning weight with increasing quantities of fish oil supplemented. It is concluded that changes in dietary lipid sources can affect the membrane's fatty acid profile within days, and mainly influences oxidative stability of the cells.

3 Dose – response effect of fish oil in parturition feed

3.1 Introduction

Fish oil, as a source of long-chain n-3 polyunsaturated fatty acids (PUFA), is frequently added to commercial gestation and lactation feeds for sows (Bimbo and Crowther, 1992). Moreover, fish oil supplementation on top of the normal feed portion is common practice during late gestation in many commercial sow herds. Although the potential benefits of fish oil supplementation on sow fertility and piglet development have been studied intensively, results are equivocal. The amount of fish oil added to the diet, the composition of the basal feed, the period within the reproduction cycle and also the duration of supplementation, vary widely between studies. Studies on the use of fish oil in sow feed mostly supplement fish oil for several weeks and compared the effect of fish oil with that of other oils providing a different fatty acid profile (Lauridsen and Danielsen, 2004; Papadopoulos et al., 2009b; Rooke et al., 1998; Rooke et al., 2000). Except for a study of Rooke et al. (2001b) and one of Fritsche et al. (1993), dose-response studies are not yet published. It can be presumed that the dietary concentration of n-3 PUFA as well as the start and duration of the supplementation will affect the outcome of such fish oil trials. For many years, research focused on the beneficial effects of additional fat in late gestation on piglet birth weight, but the specific fatty acid profile of the supplemented fat source was not taken into account (Averette et al., 1999; Cieslak et al., 1983; Seerley et al., 1981; van der Peet-Schwering et al., 2004). More recently, studies on the supplementation of specific fatty acids such as eicosapentaenoic acid (EPA, C20:5 n-3) and docosahexaenoic acid (DHA, C22:6 n-3), have been performed. These n-3 PUFA were described to affect gene transcription (Jump, 2002; Jump et al., 1996; Papadopoulos et al., 2009a), immune response (Bassaganya-Riera et al., 2007; Turek et al., 1994), fertility and reproduction (Abayasekara and Wathes, 1999; Allen and Harris, 2001; Wathes et al., 2007). It is also known that EPA and DHA are incorporated into the cell membrane, changing their fatty acid profile. The dietary fatty acid profile is also reflected in the fatty acid composition of several tissues (Haak et al., 2008; Mitchaothai et al., 2007; Straarup et al., 2006). Erythrocytes are often used as a model for cell membrane composition (Hollan, 1996; Stark, 2008). Compared with other tissue cells, erythrocytes are easier to sample and to isolate in live animals and their

composition also correlates well with the fatty acid profile of most other tissues (Fischer and Black, 1991; Mitchaothai et al., 2007; Stark, 2008; Straarup et al., 2006). Changing cell membrane composition subsequently alters several membrane-related characteristics such as osmotic fragility and oxidative stability. Osmotic fragility, defined as the resistance of erythrocytes to hypotonic shock, is determined by the sodium chloride concentration at which 50 % haemolysis occurs (Ehrstrom et al., 1981; Mineo and Hara, 2005; Mineo and Hara, 2007; Ng et al., 2001; Penha-Silva et al., 2007). Several authors report the influence of PUFA on the osmotic resistance of erythrocytes in humans (Hagve et al., 1991a; Hagve et al., 1993), rats (Hagve et al., 1991b), sheep (Shand and Noble, 1981), rabbits (Kogawa et al., 1998; van den Berg et al., 1991) and tilapia (Ng et al., 2001). However, no similar studies have been reported in pigs or sows. Besides the osmotic fragility, also oxidative stability, determined as the susceptibility of the erythrocyte membrane to different concentration of an oxidant (Stagsted and Young, 2002), can be altered by the fatty acid profile of the cell membrane. Higher amounts of PUFA in the membrane result in a higher susceptibility to free radical attack and lipid peroxidation. This leads to a higher nutritional need for antioxidants as described in rabbits (van den Berg et al., 1991), broilers (Febel et al., 2008) and piglets (Sarkadi-Nagy et al., 2003). The aim of the present study was to investigate whether n-3 PUFA are incorporated into erythrocyte membranes in a dose-responsive manner and whether this altered the cell membrane characteristics when supplied through the diet during the peripartal period of sows. To this end, different levels of n-3 PUFA were included in the diet of peripartal sows and their effects on the fatty acid profile of the erythrocyte membranes, as well as the influence on erythrocyte osmotic fragility and oxidative stability, and on sow and piglet performance were investigated.

3.2 Materials and Methods

The experiment was approved by the Ethical Committee of the Faculty of Veterinary Medicine, Ghent University, Belgium (EC 2008/048) and by the Federal Public Service Health, Food Chain Safety and Environment, Belgium.

3.2.1 Animals, experimental design and dietary treatment

The study took place at the experimental study farm of a feeding company (AVEVE N.V., Leuven, Belgium) during the months May and June 2008. The herd practiced a 3-week batch production system for the sows with approximately 28 sows/group. Two subsequent groups of sows (26 and 31 sows, respectively; average parity 4.6 ± 3.0) of the 3-week batch system were included in this study. The sows (Rattlerow Seghers hybrid) were housed in mechanically ventilated stables with a 12 h light period. From weaning until day 28 of gestation, the sows were kept individually in crates and were fed a standard gestation diet (Table 3.1). Thereafter, they were moved to a group housing system where they had *ad libitum* access to a high fibre gestation diet (Table 3.1). At day 105 of gestation, sows were transferred from the gestation unit to the farrowing unit where they were housed in individual farrowing crates until weaning (approximately 27 days after farrowing).

Table 3.1 Feeding period and feed analyses of the different feeds provided to the sows before and after the trial

	Gestation feed (standard)	Gestation feed (*ad libitum*)	Lactation feed (standard)
Feeding period[1]	Insemination → day 28	Gestation day 29 → day 109	Lactation day 3 → weaning
Dry matter[2]	885	908	911
Ash[2]	62	63	58
Crude protein[2]	135	136	161
Crude fat[2]	41	34	51
Crude fibre[2]	77	62	60
NE[3]	8.7	8.0	9.5
% Fish oil[4]	0.5	0.5	0.5
Vitamin E[5]	115	90	115

[1] Period of reproduction cycle during which the particular feed is given to the sows.
[2] Expressed as g/kg feed.
[3] Net energy (NE), calculated using feed formulation software.
[4] % on weight basis.
[5] Expressed as mg/kg feed.

The sows of each group were randomly allocated to five dietary treatment groups. One sow of the first group and five sows of the second group were excluded from the trial because they were inseminated more than 5 days earlier or later than the average insemination date of the group. For all five treatment groups (F0, F1, F2, F3, and F4), a parturition feed, low in crude fat content, was formulated to meet the sow requirements (Table 3.2).

Table 3.2 Composition of the parturition feed, without supplementation with fish oil and/or pork lard

Ingredient	%	Ingredient	%	Ingredient	%
Soybean meal	18.40	Tapioca	5.00	Linseed	0.44
Barley	15.00	Wheat gluten feed	3.45	Choline chloride	0.17
Sugar beet pulp	15.00	Maize	2.50	Vitamin premix[3]	0.08
Wheat	13.20	Limestone	1.74	Methionine	0.05
Wheat bran	10.00	Mineral premix[2]	1.09	Luctarom S[4]	0.05
Soy hulls	6.78	Soy oil	0.51	Trace elements[5]	0.02
Molasses[1]	6.00	Industrial salt	0.50	L-threonine	0.02

[1] Sugar beet molasses.
[2] Mineral premix: 4.8% mono iron sulphate, 0.5% copper sulphate, 85.3% magnesium phosphate, 6.8% monocalcium phosphate, 2.6% zinc sulphate.
[3] Vitamin premix: 6.33 g/kg vitamin A, 272.15 g/kg vitamin E, 0.063 g/kg vitamin D3, 5.02 g/kg vitamin K, 75.30 g/kg vitamin PP, 5.02 g/kg vitamin B1, 25.10 g/kg vitamin B2, 37.65 g/kg vitamin B3, 15.06 g/kg vitamin B6, 7.53 g/kg vitamin B9, 0.08 g/kg vitamin B12, 0.75 g/kg vitamin H.
[4] Luctarom S is an industrial flavour, added to the feed to improve feed intake. The product has no nutritional value.
[5] Trace elements premix: 485.07 g manganese/kg premix, 1.02 g cobalt/kg premix, 12.03 g iodine/kg premix, 2.00 g selenium/kg premix.

Table 3.3 Composition of the parturition feeds (F0 - F4) after supplementation with the different amounts of fish oil and/or pork lard

	F0	F1	F2	F3	F4
Percentage of fish oil and pork lard added to the different diets (weight base)					
% Fish oil	0	1	2	3	4
% Pork lard	4	3	2	1	0
Nutrient compostition of the diet					
Dry matter (g/kg feed)	885.1	886.6	886.0	885.0	888.8
Ash (g/kg feed)	65.6	68.8	69.9	74.6	73.5
Crude protein (g/kg feed)	156.2	157.4	157.7	153.7	155.4
Crude fat (g/kg feed)	76.5	76.1	76.7	76.0	73.3
Crude fibre (g/kg feed)	77.3	75.3	75.3	76.4	74.7
NE (MJ/kg feed)[1]	9.5	9.5	9.5	9.5	9.5
Fatty acid profile (mg fatty acids/ 100 g feed sample)					
SFA[2]	1854.8	2096.4	1999.6	2002.9	1827.6
MUFA[3]	2573.1	2675.9	2318.1	2101.9	1637.7
n-6 PUFA[4]	1720.1	1729.5	1586.4	1473.9	1339.5
$C20{:}5n{-}3$[5]	0.5	155.1	321.9	487.3	636.3
$C22{:}6n{-}3$[6]	0.6	114.5	236.2	356.5	466.7
n-3 PUFA[7]	336.7	646.5	950.0	1265.9	1527.6
n-3/n-6 PUFA[8]	0.196	0.374	0.599	0.859	1.140

[1] Net energy, calculated value using feed formulation software.
[2] Sum of all saturated fatty acids.
[3] Sum of all monounsaturated fatty acids.
[4] Sum of all n-6 polyunsaturated fatty acids (C18:2, C18:3, C20:2, C20:3 and C20:4).
[5] Eicosapentaenoic acid (C20:5 n-3).
[6] Docosahexaenoic acid (C22:6 n-3).
[7] Sum of all n-3 polyunsaturated fatty acids (C18:3, C20:3, C20:5, C22:5 and C22:6).
[8] Ratio n-3 to n-6 polyunsaturated fatty acids.

The basal diet, supplied as a crumbled pellet, was supplemented with 4 % pork lard (F0), or with 1 %, 2 %, 3 %, or 4 % of the pork lard substituted by an equal amount of fish oil (F1, F2, F3, and F4, respectively). Feed samples of each diet

were taken and analyzed according to Association of Official Analytical Chemists (AOAC) methods (Thiex, 2002) and the fatty acid profile was determined as described by Raes et al. (2001) (Table 3.3).

Dietary treatments were started at day 110 of gestation (day 1 of trial) for nine consecutive days. During the first 2 days of the trial, the sows received 2.5 kg feed/day, divided into two portions. From day 3, until day 5 of the trial, daily feed portions were diminished by 0.5 kg/day until parturition and at the actual parturition day 1.0 kg of feed/day was supplied; after parturition, the amount of feed was increased by 0.5 kg/day until day 9 of the trial. From then onwards, all sows received a commercial lactation feed until weaning that was identical for all sows (Table 3.1).

Before, during and after the experiment, all sows had free access to water.

3.2.2 Blood sampling, erythrocyte and plasma collection

On day 109 of gestation, 1 day before the feeding trial was started (day 0), and 10 days later (day 10), a 9 ml blood sample of each sow was taken from the vena jugularis in K_3EDTA tubes. Samples were stored on ice water (4 °C) until analysis. The blood samples were subsequently divided into a subsample of 1 ml and a subsample of 8 ml. The 1 ml subsample was used for the determination of osmotic fragility and oxidative stability of erythrocytes as described below. The subsample of 8 ml was centrifuged for 10 min at 1000 × g in order to separate erythrocytes from plasma and white blood cells. The blood plasma was stored at -20 °C until further analysis. Subsequently, the layer of white blood cells was removed and erythrocytes were stored at -20 °C until further analysis.

3.2.3 Fatty acid profile of erythrocytes

Five sows from the F0, F2, and F4 treatment groups were randomly selected and the fatty acid profile of erythrocytes was determined on day 0 and 10 of the trial for these sows. Fatty acids were extracted from erythrocytes using a chloroform:methanol mixture (2:1 vol/vol). After methylation, an internal standard was added and the fatty acid methyl esters (FAME) were analyzed by gas-liquid chromatography, as described in detail by Raes et al. (2001).

3.2.4 Osmotic fragility and oxidative stability of erythrocytes

Osmotic fragility was measured using hypotonic saline solutions (Penha-Silva et al., 2007; Roth and Kirchgessner, 1992). Solutions of different sodium chloride

concentration (0.0, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, and 1.0 g/dl) were made, starting from a stock solution containing 1% sodium chloride. Blood samples were analyzed in duplicate, so for each blood sample two rows of wells of a 96-well V-bottom microtiter plate were filled with 200 µl of the different sodium chloride concentrations. After incubation (10 min at 38 °C), 2 µl of whole blood was added to each well and incubated for 20 min at 38 °C. Subsequently, plates were centrifuged (1300 × g for 10 min at 38 °C), 100 µl supernatant was transferred to a flat bottom microtiter plate and absorbance was measured at 550 nm, using a Multiskan MS plate reader (Labsystems, Helsinki, Finland).

Oxidative stability was measured using a technique based on Stagsted and Young (2002). A 100 µl subsample of whole blood was diluted 50 times with phosphate buffered saline and 100 µl of this mixture was pipetted into a U-bottom microtiter plate. Samples were analyzed in duplicate, so two rows of a 96 well U-bottom microtiter plate were loaded with one blood sample. Subsequently, a series of H_2O_2 dilutions was prepared (0.00, 0.05, 0.10, 0.15, 0.20, 0.25, 0.30, 0.35, 0.40, 0.45, and 0.50 M H_2O_2) and 100 µl of each different H_2O_2 concentration was added to the 100 µl diluted blood sample in the wells. After incubation for 3 h at room temperature, plates were centrifuged (900 × g for 3 min at room temperature) and 50 µl of the supernatant was transferred to a flat bottom microtiter plate to measure absorbance at 405 nm, using a Multiskan MS plate reader (Labsystems, Helsinki, Finland).

Absorbance results of osmotic fragility and oxidative stability tests were corrected by subtracting mean blank values measured on spare wells of each flat bottom plate. The percentage haemolysis was calculated using the following formula:

$$\% \text{ haemolysis} = A / A_{max} \times 100$$

where A is the corrected measured absorbance at 550 nm for the osmotic fragility or at 405 nm for oxidative stability, and A_{max} is the mean maximal absorbance.

The concentration at which 50 % of erythrocytes were lyzed (H_{50}) was calculated from a fit of data to a sigmoid curve, using SigmaPlot software (Systat Software GmbH, Erkrath, Germany). The sigmoid equation for osmotic fragility was based on Penha-Silva et al. (2007):

$$y = y_0 + a / (1 + e^{-(x - x0)/b})$$

with y the % haemolysis, y_0 the mean minimal percentage of haemolysis, a the difference between mean maximal and mean minimal haemolysis, x the sodium chloride concentration, x_0 the sodium chloride concentration that results in 50% haemolysis ($H_{50}OF$) and b an empirical parameter indicating the steepness of the curve. All parameters in the equation were calculated using the SigmaPlot software (Systat Software GmbH, Erkrath, Germany).

For oxidative stability the sigmoid equation was based on Stagsted and Young (2002):

$$y = a / (1 + e^{-(x - x0)/b})$$

with y the % haemolysis, a the difference between mean maximal and mean minimal haemolysis, x the H_2O_2 concentration, x_0 the H_2O_2 concentration which results in 50% haemolysis ($H_{50}OS$) and b an empirical parameter indicating the steepness of the curve. All parameters in the equation were calculated using the SigmaPlot software (Systat Software GmbH, Erkrath, Germany).

3.2.5 Oxidative parameters in plasma

Oxidation of erythrocyte membrane lipids was measured as described by Grotto et al. (2007) in plasma samples of each sow and expressed as thiobarbituric acid reactive substances (TBARS). For these measurements malondialdehyde (MDA) was used as a standard and its presence after plasma sample preparation was measured using a spectrophotometer. To determine the antioxidant power of plasma, the ferric reducing ability of plasma (FRAP) assay, performed as reported by Benzie and Strain (1996), was used. For both TBARS and FRAP measurements five paired plasma samples (one sample before and after treatment of the same sow) of each treatment group were randomly selected.

3.2.6 Body condition and reproductive parameters

As an indication for sow condition, back fat thickness was measured for each sow using a Renco Lean-Meater (S.E.C. Repro Inc., Ange-Gardien-de-Rouville, Québec, Canada) on the P2 position (Maes et al., 2004). Measurements were performed on the same day as blood sampling (day 0 and day 10 of the trial). Sows' reproductive parameters (gestation length, number of piglets born, number of live born piglets, number of stillborn piglets, number of weaned piglets and percentage of dead piglets during lactation), as well as piglet performance (litter and mean individual piglet weight at birth and at weaning), were recorded.

3.2.7 Statistical analysis

The effect of increasing dietary fish oil levels on blood and reproductive parameters was tested by linear regression analyses. Differences between treatments were tested using analyses of variance (ANOVA) followed by the post-hoc Tukey test for comparison of the means. Changes over time, before and after treatment, for blood parameters and for sow body condition were determined by calculating differences between values after and before treatment and subsequently analyzed statistically as described before. To investigate the relation between oxidative stability of erythrocytes and the oxidative parameters determined in plasma (TBARS and FRAP), Pearson correlations were determined. For all statistical analyses the SPSS 16.0 software (SPSS Inc., Chicago, Illinois, USA) was used and significance was considered when $P < 0.05$. Results in the text are reported as mean ± standard deviation (SD), results in the tables are reported as mean values with the pooled standard error of the means (SEM).

3.3 Results

3.3.1 Fatty acid profile of erythrocytes

The fatty acid profile of erythrocyte membranes after different substitution levels of pork lard by fish oil, as well as the shift in profile throughout the peripartal period is given in Table 3.4.

The amount of both EPA and DHA linearly increased in the erythrocyte membranes with increasing amounts of fish oil in the diet. Also, the change in fatty acid profile caused by the dietary treatment was linearly related to the amount of fish oil supplemented, but it was more pronounced for EPA ($P < 0.001$) than for DHA ($P = 0.064$). There was also a significant linear relationship between the substitution levels and the total amount of n-3 PUFA. When comparing the change over time, the total amount of n-3 PUFA was numerically higher with increasing amounts of fish oil in the diet ($P = 0.056$). In addition to a shift in the amount of n-3 PUFA present, a shift in the amounts of saturated and monounsaturated fatty acids (SFA and MUFA, respectively) was recorded. In contrast, the total amount of n-6 PUFA was not significantly affected by substitution of pork lard by fish oil.

Table 3.4 Results of the fatty acid profile of erythrocyte membranes of sows supplemented with different amounts of fish oil in the peripartal diet (F0, F2, F4)

	F0 (0:4)[9]	F2 (2:2)	F4 (4:0)	SEM	Dose[10]
Fatty acid profile after treatment[1]	n = 5	n = 5	n = 5		
SFA[2]	34.4[11]	35.4	34.7	3.4	N.S.
MUFA[3]	27.6^a	24.0^b	22.7^b	7.4	L**
n-6 PUFA[4]	15.3	15.7	14.5	4.8	N.S.
EPA[5]	1.1^a	2.0^b	2.2^b	0.2	L**
DHA[6]	1.2^a	1.5^{ab}	1.7^b	0.1	L**
n-3 PUFA[7]	4.7^a	6.1^b	6.4^b	0.3	L**
n-6 PUFA/n-3 PUFA[8]	3.3^a	2.6^b	2.3^b	0.1	L***
Shift in fatty acid profile[1] (level at day 10 of the trial minus level at day 0 of the trial)					
SFA[2]	0.7	-0.3	-3.2	0.8	L*
MUFA[3]	0.9^a	-1.3^b	-1.8^b	0.4	L**
n-6 PUFA[4]	-0.004	-1.0	-1.3	0.8	N.S.
EPA[5]	-0.9^a	0.8^b	1.1^b	0.2	L***
DHA[6]	0.02	0.5	0.5	0.1	N.S.
n-3 PUFA[7]	-0.2	1.4	1.5	0.4	N.S.
n-6 PUFA/n-3 PUFA[8]	0.04	-1.3	-1.6	0.4	N.S.

Different letters in one row indicate significant differences between treatments (p < 0.05) determined by a post hoc Tukey test.
[1] Fatty acid profile expressed as g/100 g total fatty acids.
[2] Sum of all saturated fatty acids.
[3] Sum of all monounsaturated fatty acids.
[4] Sum of all n-6 polyunsaturated fatty acids (C18:2, C18:3, C20:2, C20:3 and C20:4).
[5] Eicosapentaenoic acid (C20:5 n-3).
[6] Docosahexaenoic acid (C22:6 n-3).
[7] Sum of all n-3 polyunsaturated fatty acids (C18:3, C20:3, C20:5, C22:5 and C22:6).
[8] Ratio n-6 to n-3 polyunsaturated fatty acids.
[9] Ratio of fish oil to pork lard supplemented in the diet.
[10] Parameters which are linearly changing with increasing or decreasing dose of fish oil supplemented are indicated with the letter L (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$), when no significant linear correlation is present the parameter is marked as not significant (NS).

3.3.2 Osmotic fragility and oxidative stability of erythrocytes

Adjusted R squares (Adj R^2) of sigmoid curve estimation of osmotic fragility tests were 0.96 ± 0.03 for curves estimated on blood samples before treatment and 0.95 ± 0.08 for curve estimation after dietary treatment. Osmotic fragility (Table 3.5) was not significantly affected by the different dietary treatments and there was no linear relation with the amount of fish oil in the diet.

Table 3.5 Results of osmotic fragility (OF) tests of the erythrocytes of sows supplemented with different amounts of fish oil in the peripartal diet (F0 - F4)

Osmotic fragility	F0 (0:4)[2]	F1 (1:3)	F2 (2:2)	F3 (3:1)	F4 (4:0)	SEM	Dose[3]
After treatment	n = 9	n = 11	n = 9	n = 11	n = 11		
$H_{50}OF$[1]	0.49	0.49	0.45	0.46	0.49	0.01	N.S.
Change over time (level at day 10 of the trial minus level at day 0 of the trial)							
$H_{50}OF$[1]	0.009	-0.002	-0.041	-0.012	-0.015	0.008	N.S.

[1] Concentration of NaCl (g/dl) at which 50% haemolysis occurs.
[2] Ratio of fish oil to pork lard supplemented in the diet.
[3] When no significant linear correlation between dose and osmotic fragility was present, the parameter is marked as not significant (NS).

For all dietary treatments including fish oil (F1, F2, F3, and F4), osmotic fragility slightly increased over time in contrast to the dietary treatment without fish oil

(F0) where a minor decrease in osmotic fragility was recorded. Hence, these differences were only numerical and not significant.

In contrast to the results of the curve estimation of osmotic fragility, the estimation for the oxidative stability tests were more variable. Curve estimation of oxidative stability measurement was not possible for six blood samples taken before the onset of the trial (one sample of F0 and of F1 and two samples of F2 and F4), and for two samples after dietary treatment (one sample of F2 and F3). Adjusted R^2 of oxidative stability averaged 0.65 ± 0.28 and 0.75 ± 0.22, before and after treatment, respectively. Sigmoid curves with an Adj R^2 lower than 0.40 were excluded from the results, leading to the exclusion of 10 samples taken before (one of F1, F3, and F4, two of F2 and five of F0), and three samples taken after dietary treatment (one of F0, F1, and F2). The limiting value of 0.40 for the Adj R^2 was determined arbitrarily, taking into account the visual evaluation of the sigmoid curve fit through each series of data. This resulted in an Adj $R^2 = 0.77 \pm 0.14$ for analyses before the onset of the trial and Adj $R^2 = 0.78 \pm 0.17$ for values after dietary treatment. There was a decrease in oxidative stability with increasing amounts of fish oil in the diet, the $H_{50}OS$ linearly decreased with increasing amounts of fish oil added to the diet (Table 3.6). Because a valid measurement before and after dietary treatment was available for only 28 out of 51 sows, no linear regression analysis was performed for the change of this parameter over time.

Table 3.6 Results of oxidative stability (OS) tests on erythrocytes and oxidative parameters measured in plasma after supplementation of the peripartal diet (F0 - F4) of sows with different amounts of fish oil

	F0 (0:4)[4]	F1 (1:3)	F2 (2:2)	F3 (3:1)	F4 (4:0)	SEM	Dose[5]
Oxidative stability	n = 8	n = 10	n = 7	n = 9	n = 11		
$H_{50}OS$[1]	0.23	0.24	0.12	0.14	0.16	0.02	L*
Oxidative parameters	n = 5	n = 5	n = 5	n = 5	n = 5		
TBARS[2]	6.0	5.8	7.2	8.8	8.3	0.5	L*
FRAP[3]	0.18	0.19	0.22	0.20	0.22	0.01	L*

[1] Concentration of H_2O_2 (M) at which 50% haemolysis occurs.
[2] Thiobarbituric acid reactive substances (TBARS) expressed as the amount of malondialdehyde (MDA) measured in plasma (nmol/ml).
[3] Ferric reducing ability of plasma (FRAP) expressed as the concentration of Fe^{2+} measured in plasma (mmol/l).
[4] Ratio of fish oil to pork lard supplemented in the diet.
[5] Parameters which are linearly changing with increasing or decreasing dose of fish oil supplemented are indicated with the letter L (* $P < 0.05$).

3.3.3 Oxidative parameters in plasma

Both FRAP and TBARS linearly increased with increasing amounts of fish oil present in the dietary treatment (Table 3.6). Neither FRAP nor TBARS showed a

significant linear regression on the differences over time for the different dietary treatments (data not shown). Also, no significant correlations were found between the oxidative stability and both oxidative parameters determined in plasma (data not shown).

3.3.4 Sow and piglet performance

The reproductive and performance results of the sows and piglets are presented in Table 3.7. The back fat thickness significantly diminished for all sows during the peripartal period, but there was no significant influence of treatment ($P >$ 0.05). The feed intake of the sows in the different treatment groups was similar throughout the trial. The gestation length was not influenced by the treatment ($P > 0.05$). There was a slight numerical increase in number of live born piglets with increasing amount of fish oil in the diet. The number of stillborn piglets was not significantly different between treatments.

Table 3.7 Results of sow and piglet performance when sows were fed a diet with different amounts of fish oil during the peripartal period

	F0 (0:4)[3]	F1 (1:3)	F2 (2:2)	F3 (3:1)	F4 (4:0)	SEM	Dose[4]
	n = 9	n = 11	n = 9	n = 11	n = 11		
Parity	4.1	4.6	4.9	4.5	4.9	0.4	
Back fat loss (mm)	1.4	1.7	3.6	1.6	1.0	0.4	N.S.
Gestation length (days)	116.4	116.1	115.7	115.6	116.9	0.2	N.S.
Total litter size	12.4	12.7	13.2	13.3	13.6	0.4	N.S.
Live born piglets	11.0	11.6	11.0	12.1	12.6	0.5	N.S.
Stillborn piglets	1.4	1.1	2.2	1.2	1.1	0.3	N.S.
Weaning number	9.8	9.6	9.9	10.5	9.7	0.3	N.S.
Piglet mortality (%)	7.4	11.0	14.2	17.1	18.4	1.9	L*
Birth weight (kg)[1]	1.4	1.6	1.4	1.4	1.4	0.1	N.S.
Weight gain (g/day)[2]	252	231	238	249	223	7	N.S.
Weaning weight (kg)	8.5	7.4	8.2	7.7	7.0	0.2	L*

[1] Mean birth weight per piglet only recorded from 25 of 51 litters (F0: 4, F1: 7, F2: 3, F3: 7 and F4: 4).
[2] Average weight gain per piglet throughout lactation calculated for 25 of 51 litters (F0: 4, F1: 7, F2: 3, F3: 7 and F4: 4).
[3] Ratio of fish oil to pork lard supplemented in the diet.
[4] Parameters that are linearly changing with increasing or decreasing dose of fish oil supplemented are indicated with the letter L (* $P < 0.05$), when no significant linear connection is present the parameter is marked as not significant (NS).

Mean individual birth weight of live born piglets was not significantly influenced by treatment. This parameter was recorded only for sows that farrowed between 7.00 and 19.00 h (i.e. 25 out of 51 litters) because there was no supervision during the night. Mean daily weight gain of piglets throughout the entire lactation (26 ± 2 days) for these 25 litters was 239 ± 36 g/day and no significant relation with amount of fish oil in the diet was recorded. The mean individual weaning

weight decreased and the percentage of piglets that died throughout lactation increased linearly with increasing amounts of fish oil in the diet.

3.4 Discussion

Results of the present study clearly show that the fatty acid profile of erythrocytes can be altered, even after a short period of fish oil supplementation. These results implicate that specific PUFA in the diet are not only absorbed well from the feed, as described before by Innis and Dyer (1999) for neonatal piglets, but are actually incorporated in the cell membranes. Considering the fact that the lifetime of erythrocytes is approximately 72 days (Withrow and Bell, 1969), the rather quick response of the cell membrane fatty acid profile implicates that n-3 PUFA are incorporated into the erythrocyte membranes after cell formation. Moreover, both EPA and DHA, the two most important n-3 PUFA present in fish oil, increased in a dose-responsive manner in the erythrocyte membranes. The same statement holds for the total amount of n-3 PUFA. Dose-response studies with fish oil supplementation in sows are scarce. Rooke et al. (2001b) supplemented 0, 0.5, 1.0 and 2.0% of fish oil to sow diets from day 60 of gestation until parturition, but in contrast to the present study, only plasma samples were analyzed to determine the fatty acid profile. Also Fritsche et al. (1993) determined only the fatty acid profile of serum and milk of sows supplemented with 0, 3.5 and 7% of fish oil from day 107 of gestation until weaning. Most other studies in pigs have focused on the fatty acid profile of adipose and muscular tissue of fattening pigs, in order to alter the fatty acid profile of the meat (Nguyen et al., 2003). Interestingly, both Straarup et al. (2006) and Mitchaothai et al. (2007) reported that the fatty acid profile of erythrocyte membranes mirrors that of the dietary fat. Results of the present study agree with these latter studies, except for the fact that in the present study the supplementation period was much shorter and that different types of fat sources were used. Besides the studies performed in pigs, results of studies performed in other species, i.e. mice (Fischer and Black, 1991) and humans (Damsgaard et al., 2007; Hagve et al., 1991a; Hagve et al., 1993; Harris et al., 2007) corroborate with the findings of the present study. All latter studies reported an increase of n-3 PUFA in the erythrocyte membranes when fish oil is added to the diet.

Although fish oil supplementation resulted in a dose-responsive change in the fatty acid profile of erythrocyte membranes, osmotic fragility of erythrocytes was not affected. Meaning that supplementation of up to 4 % fish oil to sows diet does not affect the osmotic fragility of erythrocyte membranes. The results of the present study are in line with results from a study in mice (Fischer and Black, 1991) and in tilapia (Ng et al., 2001), both reporting no changes in osmotic fragility after supplementing n-3 or n-6 PUFA during 11 and 8 weeks, respectively. However, studies in rats (Hagve et al., 1991b) and humans (Hagve et al., 1991a; Hagve et al., 1993) reported decreased osmotic fragility after fish oil supplementation for one to three weeks. When either n-6 PUFA (Huang et al., 1983; Roth and Kirchgessner, 1991) or n-3 PUFA (Roth and Kirchgessner, 1992) were supplemented to rats deficient in essential fatty acids, the osmotic fragility of the rat erythrocytes decreased.

The results for the oxidative stability of erythrocytes in the present study were consistent with the predicted values for pig erythrocytes as described by Stagsted and Young (2002). Except for the latter study, no other studies with a similar testing method have yet been published. Two other parameters used in similar studies investigating the effect of n-3 PUFA on oxidative stability of cell membranes are glutathione peroxidase (GPx) and superoxide dismutase (SOD) activity. Studies in several animal species have indicated that both SOD and GPx activity are reliable parameters to measure oxidative stability of erythrocytes caused by dietary PUFA (De Moffarts et al., 2007; Erdogan et al., 2004; Trenzado et al., 2009). Although these two latter reported parameters were not determined in this trial, they could provide useful information in addition to the oxidative stability test performed in the present study.

The decreased oxidative stability of erythrocytes with increasing amounts of n-3 PUFA in the diet was confirmed by the results of TBARS. In the present study lipid peroxidation increased with increasing amounts of n-3 PUFA in the diet and concomitant higher n-3 PUFA in the erythrocytes. This in accordance with findings of Perez-Mateos et al. (2005) reporting a significant increase of TBARS in rat plasma after a 3-week supplementation of the diet with fish oil but not after a 3-week supplementation of the diet with sunflower oil. Similarly, Ebeid et al. (2008) recently concluded that TBARS measured in plasma increased in a dose-responsive manner when fish oil was supplemented for 12 weeks to laying hens. In contrast with these latter study results, Sarkadi-Nagy et al. (2003)

reported that there is no influence of the amount of DHA present in the diet of piglets. Two other studies on fish oil supplementation in the diet of rats even reported the opposite effect on TBARS (Carrapeiro et al., 2007; Erdogan et al., 2004). Based on the increasing TBARS levels in plasma with increasing amounts of fish oil in the diet of the present study, it was expected that the antioxidant capacity of the plasma would decrease with increasing amounts of fish oil supplemented. However, our results indicated the opposite: FRAP values increased with increasing amounts of n-3 PUFA in the diet and consequently also in the erythrocytes. This apparent contradiction between TBARS and FRAP can be explained by the hypothesis that with increased lipid peroxidation, antioxidant mechanisms of the sow are activated which resulted in higher FRAP levels when higher portions of fish oil were present in the diet. A lack of positive correlation between TBARS and FRAP measurements is also described in other studies (Hadi et al., 2004; Mudron et al., 2007).

As no difference in feed intake was observed between the different treatment groups, it can be presumed that addition of fish oil caused no negative taste influence for the sows. Seen the equal energy content of all five diets this could be an explanation for the similar back fat loss of all sows throughout the trial.

Even though the dietary treatment stopped on day 3 of lactation, increasing amounts of fish oil in the peripartal diet of the sow seemed to have a negative influence on piglet performance throughout lactation. Seen the absence of clinical parameters of the piglets, no clear explanation for these results could be given and further research on this topic is advisable. Except for piglet survival and piglet weaning weight, fish oil supplementation did not affect sow and piglet performance parameters. These findings are in line with results of both Fritsche et al. (1993) and Rooke et al. (2001b).

In conclusion, different substitution levels of pork lard by fish oil during the peripartal period resulted in actual incorporation of n-3 PUFA into the erythrocytes, making them available to take part in various physiological processes. Despite the dose-responsive incorporation of n-3 PUFA, no effect on erythrocyte osmotic fragility could be detected. In contrast, the oxidative stability of erythrocytes decreased with increasing amounts of n-3 PUFA present in the cell membrane. Results of the oxidative stability test were confirmed by increased lipid peroxidation in plasma with increasing amounts of fish oil in the

diet. This was accompanied by increased antioxidant capacity of the plasma, probably due to up-regulation of antioxidant pathways.

The results of the present study implicate that although n-3 PUFA were incorporated in the erythrocytes, osmotic resistance of erythrocytes was not altered, whereas oxidative stability of erythrocytes, as well as oxidative parameters in plasma, were affected by the dose-responsive incorporation of n-3 PUFA. These findings should be considered in practice, seen the importance of membrane stability in the susceptibility of several tissues for metabolic disorders.

Acknowledgements

Chapter 4

Feed intake regulating hormones in peripartal sows

Peripartal changes in orexigenic and anorexigenic hormones in relation to back fat thickness and peripartal feeding strategy of sows

A. Cools, D. Maes, R. Decaluwé, J. Buyse, T.A.T.G. van Kempen, G.P.J. Janssens

Highly prolific sows often suffer from peripartal hypophagia, resulting in a decreased production rate. Leptin, ghrelin, and resistin are known as feed intake regulating hormones in many species, but it is yet unknown how feeding strategy and body condition will affect these hormones around parturition in sows. In the present study, a total of 63 parity two to seven sows were divided over two treatment groups which were fed either restricted (RESTRICT) or ad libitum *(ADLIB) during the peripartal period (day 106 of gestation until day 7 of lactation). Within each treatment group, sows were assigned to one out of three body condition groups based on back fat thickness at day 106 of gestation: less than 18 mm (LEAN), between 18 and 22 mm (MODERATE), and more than 22 mm (FAT). Postprandial blood samples were taken on days 107, 109, and 112 of gestation and on days 1, 3, and 5 of lactation. Using RIA, leptin, ghrelin, and resistin of each sample was analyzed. For both leptin and resistin, the hormonal profile gradually increased throughout the peripartal period (P < 0.001) whereas ghrelin peaked on day 109 of gestation as compared with day 107 of gestation and day 1 of lactation. Other time points were intermediate between those two (P = 0.012). The peripartal profile of leptin was significantly higher for FAT sows in comparison to the two other condition groups. No effect of body condition on ghrelin and resistin levels was observed. None of the three measured hormones were affected by feeding strategy. In conclusion, during the peripartal period feed intake of sows did not affect leptin, ghrelin, or resistin profiles. Leptin was the only hormone investigated that reflected body condition. Although body condition and late gestation feed intake have been previously described as risk factors for peripartal hypophagia, they did not induce hypophagia in any of the sows or affect the profile of the observed feed intake regulating hormones during the peripartal period.*

4 Feed intake regulating hormones in peripartal sows

4.1 Introduction

Peripartal hypophagia is a frequently occurring problem in highly prolific sows resulting in depressed milk production and increased piglet mortality during the first week of lactation (Edwards, 2002). Several risk factors, such as sows' body condition at the end of gestation or high feed intake levels during late gestation, have been identified in previous reports (Maes et al., 2010; Papadopoulos et al., 2010). However, the actual causes and physiological background for this peripartal hypophagia syndrome remains unclear. A peripartal profile of feed intake regulating hormones, which also intervene in reproduction through regulation of energy expenditure (Barb and Kraeling, 2004; Budak et al., 2006), may provide novel insight into hypophagia. Feed intake is partly regulated by means of orexigenic and anorexigenic hormones (Carroll and Allee, 2009). Most of these (an)orexigenic hormones such as leptin, ghrelin, and resistin, affect feed intake by acting on the hypothalamic appetite centers (Barb et al., 2001; Budak et al., 2006). In sows it was previously reported that the anorexigenic hormone leptin correlates well with the amount of body fat (Estienne et al., 2000; Estienne et al., 2003; Mosnier et al., 2010; Prunier et al., 2001) and the level of energy intake of the sow (Prunier et al., 2001; Quesnel et al., 2009). In contrast to leptin, ghrelin is an orexigenic hormone that is not produced by the adipose tissue but is mainly secreted by the stomach (Budak et al., 2006; Scrimgeour et al., 2008). A third, more recently discovered appetite regulating hormone, is resistin. This hormone is an adipocyte-derived polypeptide and influences appetite by increasing insulin resistance (Budak et al., 2006; Henry and Clarke, 2008). Research has pointed out that the plasma concentration of resistin is positively related to the degree of obesity of the pig (Chen et al., 2004). Apart from the feed intake regulating function of leptin, ghrelin, and resistin, these hormones also participate in the regulation of energy expenditure and can affect reproduction (Budak et al., 2006). However, little is known about the peripartal profile of leptin, ghrelin, and resistin in sows. The present study investigated the changes of these feed intake regulating hormones around the time of parturition as affected by body condition and the feeding strategy.

4.2 Materials and methods

The procedures used in the present trial were in accordance with the Polish legislation and guideliness for animal welfare and experiments.

4.2.1 Animals, feeding, and experimental design

The trial was performed at the experimental farm of Provimi Poland Sp.z.o.o. (Bieganów, Cybinka, Poland). From two subsequent production groups of the 1-week batch production system practiced at the farm, 63 parity two to seven sows (PenArLan Naïma Hybrid, Poland) were selected. From insemination (day 1 of gestation) until day 105 of gestation sows were all restricted fed following identical feeding schedule using a standard gestation diet (898.0 g/kg dry matter, 133.1 g/kg crude protein, 14.6 g/kg crude fat, 49.6 g/kg crude fibre, and 50.4 g/kg crude ash, all expressed on as-fed basis). At day 105 of gestation, after consumption of the last portion of gestation feed, sows were transferred to the farrowing unit. On days 106, 109, and 112 of gestation and on days 1, 3, and 5 of lactation back fat thickness of sows was measured at the P2 position (Maes et al., 2004) using a Renco Lean-Meater (S.E.C. Repro Inc., Ange-Gardien-de-Rouville, Québec, Canada). Selected sows were randomly assigned to one of two treatment groups, taking into account equal distribution of both parity and back fat thickness. One treatment group (RESTRICT) was fed a standard wheat-triticale-soybean meal based lactation feed (891.9 g/kg dry matter, 160.8 g/kg crude protein, 34.9 g/kg crude fat, 54.6 g/kg crude fibre, and 48.9 g/kg crude ash, all expressed on as-fed basis) following a restricted feeding schedule from day 106 of gestation until day 7 of lactation (starting at 3.2 kg, gradually decreasing by 0.3 kg daily until farrowing and increasing with 1 kg/day from farrowing onwards till *ad libitum* intake was reached). The total daily amount of feed offered was divided over three feeding portions and total feed consumed per sow was recorded daily. The other treatment group (ADLIB) was fed *ad libitum* during the peripartal period, using the same lactation diet as for the RESTRICT group. Sows of the ADLIB group were fed three times daily a portion of 3 kg to stimulate feed intake and daily feed intake of each sow was recorded. Based on back fat thickness at day 106 of gestation, each treatment group was further subdivided into three groups. Sows with back fat thickness at the P2 below 18 mm were assigned to the lean group (LEAN), those with back fat thickness above 22 mm were categorized as fat (FAT) and sows with back fat thickness between

18 and 22 mm were assigned to the group with moderate body condition (MODERATE). Selection of the back fat levels for the three body condition groups was set at the above values so body condition groups were of comparable size. Total number of piglets born (sum of live born and stillborn) was recorded and within 24 h after parturition litters were standardized to 10 ± 1 piglets of comparable litter weight. At days 1, 3, and 5 of lactation all litters were weighed. During the entire trial, all sows had *ad libitum* access to fresh drinking water.

4.2.2 Blood samplings

On days 107, 109, and 112 of gestation and on days 1, 3, and 5 of lactation a 10 ml blood sample was taken from each sow by *vena jugularis* puncture. At each sampling day, blood samples were collected between 8:00 and 10:00 a.m., which was 2 h after the morning feeding. Immediately after venopuncture, the samples were divided into two subsamples (6 ml on lithium heparine and 4 ml on serum clot activator) and stored at 4 °C for a maximum of 3 h until centrifugation (10 min, 4 °C, 1000 × *g*). Collected plasma and serum fractions were stored at -20 °C until further analyses.

4.2.3 Blood analyses

Leptin was measured in plasma samples using a multispecies RIA kit (Multi-Species Leptin RIA Kit, Linco Research, Missouri, USA) with a guinea pig anti-multispecies leptin antibody and ^{125}I-human leptin label. Cross-reaction of the antiserum with porcine leptin is reported by the manufacturer as 67% relative to human leptin. Sensitivity of the assay in our laboratory was 0.4 ng/ml. The assay has been frequently used for assay of leptin in pigs (Estienne et al., 2000; Prunier et al., 2001; Quesnel et al., 2009), and quality controls provided by the manufacturer as well as external control samples were within the expected range. Plasma samples were also analyzed for ghrelin with a porcine RIA kit (Porcine Ghrelin RIA KIT, Phoenix Peptide, California, USA) which uses a rabbit antiserum specific for the porcine ghrelin peptide and ^{125}I-porcine ghrelin label. Sensitivity of the assay was 45 pg/ml. Spiking plasma with porcine ghrelin resulted in a recovery of 96% to 114%. Resistin was determined in serum using a commercial human RIA kit (Resistin RIA KIT, Phoenix Peptide, California, USA) which included a rabbit antiserum to resistin and ^{125}I-human resistin label. Porcine resistin was not available; thus, porcine serum was spiked with human resistin, resulting in a recovery that ranged from 88 % to 125 %. Dilution of

porcine serum samples (dilution factors 1.25, 1.67, and 2) resulted in recovery that ranged from 96 % to 119 %. Sensitivity of the assay was 194 pg/ml. Standards, totals, blanks, and quality controles provided by the manufactureres of each kit were analyzed in triplicate. All samples were analyzed in duplicate and in the same run to avoid inter-assay variations. Intra-assay variation between duplicate samples was 7.8 % for leptin, 8.8 % for ghrelin, and 7.4 % for resistin.

4.2.4 Statistical analyses

All statistical analyses were performed using SPSS 21.0 (SPSS, Chicago, Illinois, USA) and statistical significance was set at $P < 0.05$. Correlations between back fat thickness, feed intake, and feed intake regulating hormones on each individual sampling day were tested by bivariate Pearson correlation. Furthermore, data were subjected to repeated measures analyses of variance (ANOVA) with time of sampling as within-subject factor and both feeding strategy and body condition as between-subject factors. Data with only one measurement in time were subjected to univariate ANOVA with feeding strategy and body condition as fixed factors. Normality of the residuals was tested using a Kolmogorov-Smirnoff test and homogenity of variance was verified using the Levene's test. When not significant, interaction terms were removed from the model. To determine significant differences between different body condition groups a post-hoc Tukey test was performed whenever appropiate. Significant differences between different time points were determined using least significant differences (LSD) test and Bonferroni correction was applied. Data were reported as mean ± standard deviation (SD) except when reported otherwise.

4.3 Results

During the peripartal period, average daily feed intake for the ADLIB group was 7.1 ± 0.9 kg/d whereas the RESTRICT group consumed 3.0 kg/d. Body condition did not affect feed intake. An overview of average parity, back fat levels, total born piglets and total litter weight is presented in Table 4.1.

Table 4.1 Overview of parity, evolution of back fat (BF), total piglets born, and evolution of litter weight of the different treatment groups.

	ADLIB[1]			RESTRICT			SEM	P-value[2]		
	LEAN[3] N = 10	MODERATE N = 13	FAT N = 8	LEAN N = 9	MODERATE N = 13	FAT N = 10		F × C	F	C
Parity	4.2	3.4	4.1	3.9	3.9	4.2	0.2	NS	NS	NS
BF gestation day 106[4, 5]	15.4[a]	20.2[b]	25.9[c]	16.0[a]	20.5[b]	24.8[c]	0.5	NS	NS	< 0.001
BF lactation day[4, 5]	16.0[a]	21.0[b]	24.7[c]	16.8[a]	20.2[b]	22.4[c]	0.5	NS	NS	< 0.001
BF change[4, 6]	0.7[a]	0.7[ab]	-1.2[b]	0.8[a]	-0.2[ab]	-1.4[b]	0.3	NS	NS	0.013
Total piglets born[4]	9.1[x]	10.8[xy]	10.9[xy]	11.6[y]	10.2[xy]	12.0[y]	0.2	0.012	0.024	NS
LW lactation day 1[7]	17.2	17.0	15.6	18.4	17.8	16.8	0.4	NS	NS	NS
LW lactation day 3[7]	20.1	19.6	17.9	20.2	21.0	20.2	0.5	NS	NS	NS
LW lactation day 5[7]	23.8	23.7	21.3	22.9	25.0	24.6	0.6	NS	NS	NS
Litter growth[8]	6.6	6.7	5.7	4.6	7.2	7.9	0.4	0.088	NS	NS

[1] Feeding strategy applied during the peripartal period (d 106 of gestation until day 7 of lactation): ADLIB were fed *ad libitum* (maximum of 9 kg) a standard lactation pellet, RESTRICT were fed the same lactation pellet following a restricted feeding scheme.
[2] *P*-values for interaction feeding strategy × body condition (F × C), feeding strategy (F), and body condition (C), when only a trend (*P* < 0.10) was observed, the value is represented between brackets and when not significant this was indicated by NS.
[3] Body condition groups based on back fat thickness (BF): LEAN BF < 18 mm, MODERATE 18 mm ≤ BF < 22 mm, FAT BF > 22mm.
[4] Results within one row with different small letters (a - c) represent differences caused by body condition, differences caused by feeding strategy × body condition interaction were indicated by small letters (x - y).
[5] Back fat thickness (BF) expressed in mm and measured at the P2 position.
[6] Change of back fat thickness (BF) between day 106 of gestation and day 5 of lactation (mm).
[7] Litter weight (LW) expressed in kg and recorded at every post partum blood sampling.
[8] Litter growth between day 1 and day 5 of lactation (kg).

For ghrelin, three sows (one RESTRICT-FAT, one ADLIB-MODERATE, and one RESTRICT-LEAN) had high ghrelin levels, more than a 10-fold increase compared to other sows. Therefore, ghrelin results of these three sows were excluded from further analyses. None of the feed intake regulating hormones were significantly correlated with back fat thickness or peripartal feeding strategy (Table 4.2), except for a minor positive correlation between back fat thickness and leptin on day 109 of gestation ($R^2 = 0.154$) and day 3 of lactation ($R^2 = 0.077$).

Table 4.2 Overview of bivariate Pearson correlations between feed intake and back fat thickness, and leptin, ghrelin, and resistin at six different time points during the peripartal period.

	Leptin		Ghrelin		Resistin	
	CC[1]	*P*-value[2]	CC	*P*-value	CC	*P*-value
Gestation d 107						
Feed intake	-0.101	NS	0.004	NS	0.025	NS
Back fat thickness	0.234	(0.070)	0.039	NS	-0.163	NS
Gestation d 109						
Feed intake	0.101	NS	-0.089	NS	-0.093	NS
Back fat thickness	0.393	0.003	0.106	NS	-0.107	NS
Gestation d 112						
Feed intake	0.120	NS	0.070	NS	-0.019	NS
Back fat thickness	0.080	NS	0.044	NS	-0.104	NS
Lactation d 1						
Feed intake	-0.028	NS	0.078	NS	0.038	NS
Back fat thickness	0.070	NS	0.066	NS	-0.140	NS
Lactation d 3						
Feed intake	-0.082	NS	-0.007	NS	0.077	NS
Back fat thickness	0.278	0.027	0.041	NS	-0.174	NS
Lactation d 5						
Feed intake	-0.065	NS	0.160	NS	0.085	NS
Back fat thickness	0.157	NS	0.054	NS	-0.122	NS

[1] Pearson correlation coefficient (CC).
[2] *P*-values of Pearson correlation analyses, when only a trend was observed (*P* < 0.10) *P*-value was reported between brackets, and when not significant NS was reported.

Table 4.3 Main effects of feeding strategy on levels of leptin, ghrelin, and resistin during the peripartal period

	ADLIB[1]	RESTRICT	SEM	*P*-value[2]
Leptin (ng/ml)	3.3	3.1	0.08	NS
Ghrelin (pg/ml)	541	535	18	NS
Resistin (pg/ml)	515	491	10	NS

[1] Applied feeding strategy during the peripartal period: *ad libitum* feeding (ADLIB) or restricted feeding (RESTRICT).
[2] *P*-values, when not significant NS was reported.

Table 4.4 Main effects of body condition on levels of leptin, ghrelin, and resistin during the peripartal period

	LEAN[1]	MODERATE	FAT	SEM	*P*-value[2]
Leptin (ng/ml)[3]	2.9[a]	3.1[a]	3.6[b]	0.08	0.003
Ghrelin (pg/ml)	554	528	535	18	NS
Resistin (pg/ml)	524	492	496	10	NS

[1] Body condition groups of sows based on back fat thickness (BF): LEAN BF< 18 mm, MODERATE 18 mm < BF < 22 m, FAT BF > 22 mm.
[2] *P*-values, when not significant NS was reported.
[3] Results labbelled with different letters within one row were significantly different.

For the measured hormones neither 3-way nor 2-way interactions between the tested factors (time, feeding strategy, and body condition) were statistically significant. Therefore, all interaction terms were removed from the models and only the main effects were kept. Sows' body condition affected leptin concentrations (P = 0.003, Table 4.4), with significantly higher postprandial leptin levels for sows of the FAT group when compared to LEAN and MODERATE, regardless of periperatal feeding strategy (P > 0.05, Table 4.3). Also sampling day significantly affected leptin concentrations (P < 0.001) resulting in a gradual increase of postprandial leptin levels throughout the peripartal period (Figure 4.1).
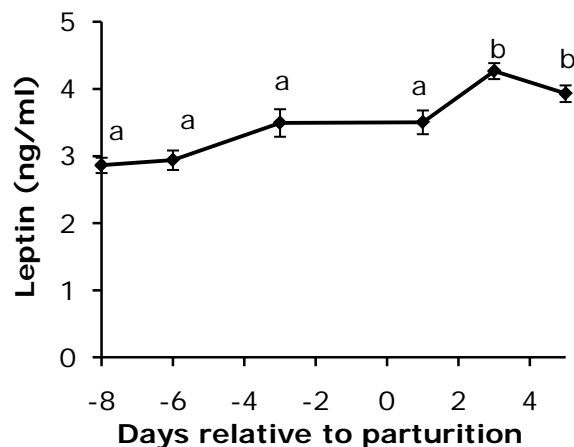
Figure 4.1 Peripartal leptin profile (mean ± SEM) of sows showed a time dependent profile (P < 0.001), time points with different letters were significantly different.

Ghrelin profile during the peripartal period was affected neither by feeding strategy (Table 4.3), nor by body condition of sows (Table 4.4). As far as the different sampling times, some significant variation in ghrelin levels could be detected (P < 0.001). The ghrelin levels at day 107 of gestation and day 1 and 3 of lactation were significantly lower than those at day 109 of gestation and day 5 of lactation (Figure 4.2). Throughout the peripartal period, resistin levels

gradually increased (P < 0.001, Figure 4.2), although, irrespectively of body condition and feeding strategy (Table 4.3 and 4.4).
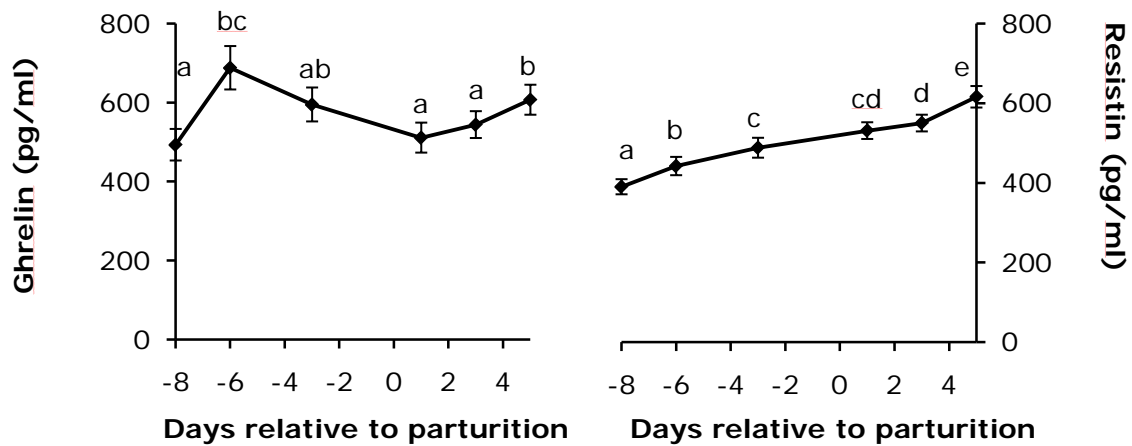
Figure 4.2 Peripartal ghrelin and resistine profile (mean ± SEM) of sows showed a time dependent profile (different letters mark significantly different time points, P < 0.001).

4.4 Discussion

For the three hormones investigated in the present trial, a time-dependent profile throughout the peripartal period was identified. As expected, the postprandial leptin concentration during the peripartal period was higher for sows with higher back fat levels. This is in line with previous research reporting elevated leptin levels at farrowing for sows with back fat thickness at the P2 exceeding 24 mm (De Rensis et al., 2005) or 25 mm (Estienne et al., 2000), compared with sows lower in back fat thickness at farrowing. These findings support the hypothesis that with increasing amounts of adipose tissue, leptin levels also increase. Leptin was not correlated with feeding strategy, which is in agreement with Prunier et al. (2001) reporting only a correlation of leptin with gilts fatness and not with feeding strategy during lactation (*ad libitum* versus restricted). Although leptin levels in FAT sows were higher, their actual feed intake was not influenced and no hypophagic sows were seen in the present trial. This could possibly be explained by the occurrence of a state of leptin resistance during the peripartal period as has been described in humans (Butte et al., 1997; Hauguel-de Mouzon et al., 2006; Moschos et al., 2002) and rats (Ladyman, 2008). With the progression of gestation the total amount of leptin produced increases resulting in decreased leptin sensitivity (Butte et al., 1997). The increase in leptin seen during the peripartal period confirms this hypothesis. Together with the lack of effect of feeding strategy during the peripartal period, it

can be assumed that leptin resistance or decreased leptin sensitivity continues shortly after farrowing. For sows (De Rensis et al., 2005; Estienne et al., 2000) and gilts (Prunier et al., 2001), as well as for other mammals such as dairy cattle (Meikle et al., 2004), goats (Rasmussen et al., 2008), horses (Berg et al., 2007), rats (Woodside et al., 2000), and humans (Butte et al., 1997) a postparturient normalization of the elevated gestational leptin levels has been reported. Our findings of increasing leptin levels shortly after farrowing are in contrast with these reports. Taking a closer look at these studies, leptin concentrations were measured less frequently around farrowing. It is, therefore, possible that leptin only decreased starting from day 3 or 4 of lactation onwards as could be observed in the present trial and in the report of Prunier et al. (2001).

To our knowledge, there is few published information on peripartal ghrelin profiles in sows. Only one study of Govoni et al. (2007) reported lower ghrelin levels for gestation and lactating sows when compared to non producing sows, without differences in ghrelin levels of sows at day 90 of gestation, compared to sows at day 7 of lactation. The present study, however, focused on the short period around farrowing and showed a rapidly changing ghrelin pattern around farrowing. Although ghrelin expression in sows relates to the estrous cycle (Zhang et al., 2008) and in humans postprandial ghrelin decreased postpartum (Larson-Meyer et al., 2010), circulating postprandial ghrelin levels during the peripartal period in sows seemed not to be affected. In contrast to the decrease of postprandial ghrelin levels with increasing intake of concentrate in dairy cattle (Roche et al., 2007), feeding strategy did not affected ghrelin concentrations in peripartal sows. This suggests that in sows, similar to growing pigs (Scrimgeour et al., 2008), ghrelin responded more to chronic changes in energy balance rather than to actual feed intake. Despite reports about the inverse relationship between ghrelin levels and adiposity in humans (Larson-Meyer et al., 2010) and sheep (Kurose et al., 2005), the link between adiposity and ghrelin levels of peripartal sows could not be demonstrated. It can thus be assumed that in peripartal sows, analogous to growing pigs (Scrimgeour et al., 2008), ghrelin only reflects the current energy status independently from adiposity. As ghrelin is mainly secreted by the stomach mucosa (Dong et al., 2009), the deviating ghrelin profile of the three individual sows, excluded from further statistical analyses, could have been due to underlying pathology of the stomach mucosa. One explanation is the occurrence of gastric ulceration, which is common to

sows. Different risk factors including infections with *Helicobacter suis*, have been described. However, reports on the effect of *Helicobacter pylori* infection in humans indicated either a decreased ghrelin production (Osawa et al., 2005), or a lack of detectable effect on ghrelin secretion (Gokcel et al., 2003). Another hypothesis is the presence of a gastric ghrelinoma as previously described in humans (Tsolakis et al., 2004). This malignant ghrelinoma could lead to hyperghrelinomia. As no postmortem information about these particular sows is available and the adipocyte derived hormones (leptin and resistin) of these sows seemed normal, no final conclusion on the actual cause of the deviating ghrelin pattern can be made.

Research in pigs, as an animal model to evaluate the effect of resistin in the pathogenesis of human insulin resistance, revealed that resistin expression pattern of pigs is similar to that of humans (Dai et al., 2006). However, while the porcine resistin gene has been characterized several years ago (Dai et al., 2006), the function of resistin in the porcine metabolism remains largely unknown. From the present study, it can be concluded that resistin increased throughout the peripartal period, regardless of adiposity of sows or applied feeding strategy. Although research in growing pigs reported lower resistin expression in adipose tissue of lean pigs and an increased expression with the development of obesity (Chen et al., 2004), more recent work of Dia et al. (2006) pointed out that resistin gene expression in leukocytes is much higher than in adipose tissue. Therefore, previous reports on resistin expression measured in adipose tissue and resistin levels measured in plasma or serum could not be compared because expression by leukocytes is underestimated. Whereas in the present study an increase of postprandial resistin levels was observed until day 5 of lactation, research in humans indicates a gradual decrease of resistin during lactation, starting from day 3 onwards (Ilcol et al., 2008) and lowered levels at 6 months postpartum (Megia et al., 2008). Again, a positive correlation between obesity and resistin production was reported (Megia et al., 2008). It is known that resistin antagonizes insulin functioning (Adeghate, 2004; Budak et al., 2006), resulting in decreased insulin sensitivity. Therefore, the observed increased resistin levels during the peripartal period could partly be responsible for a state of insulin resistance of sows. This peripartal insulin resistance leads to increased glucose half-life (Père et al., 2000; Père and Etienne, 2007; Quesnel et al., 2009), and increases the glucose levels without a

concomitant rise in insulin levels (Foisnet et al., 2010b). As a result, the mammary tissue benefits from the higher availability of glucose for the start of colostrum production and the onset of lactation (Foisnet et al., 2010b; Reynolds and Rook, 1977).

In conclusion, peripartal profiles of both ghrelin and resistin changed over time regardless of the body condition or peripartal feeding strategy of the sows. Leptin concentrations also changed over time, with higher values in fat sows but with no influence from peripartal feeding strategy. These results indicate that during the peripartal period feeding strategy did not affect the three feed intake regulating hormones determined in this trial.

Acknowledgments

Chapter 5

N,N-dimethylglycine in parturition feed

Effect of N,N-dimethylglycine supplementation in parturition feed for sows on metabolism, nutrient digestibility and reproductive performance

A. Cools, D. Maes, J. Buyse, I.D. Kalmar, J.-A. Vandermeiren, G.P.J. Janssens

The current pilot study assessed the influence of N,N-dimethylglycine (DMG) on insulin sensitivity, glucose and fat metabolism, nutrient digestibility, and reproductive performance of sows in the peripartal period. At day 105 of gestation, 25 sows were randomly assigned to the control (n = 13) or the DMG group (n = 12). Sows from the DMG group were supplemented with 1 g DMG/kg feed until day 3 of the lactation. After an overnight fast one day after farrowing, a blood sample of each sow was drawn. The plasma was analyzed for insulin, glucose, fructosamine, leptin, thiobarbituric acid reactive substances (TBARS), ferric reducing ability of plasma (FRAP), non-esterified fatty acids (NEFA) and triglycerides (TG), and an oral glucose tolerance test (OGTT) was performed. A rectal feces sample was collected and the apparent fecal digestibility (AFD) of crude fat, crude protein, and nitrogen free extract was calculated after proximate analyses. Finally, a colostrum sample was collected from each sow and analyzed for the presence of DMG. Reproductive performance parameters were recorded. The results showed an improvement in AFD of crude fat, crude protein, and nitrogen-free extract when DMG was supplemented. This beneficial effect confirms the hypothesis that DMG acts as an emulsifying agent. The improvement in digestibility in the DMG group was accompanied by a numerical increase of plasma TG (P = 0.067). Plasma NEFA concentrations were not different between treatment groups. DMG supplementation neither affected glucose clearance nor influenced plasma insulin, glucose, fructosamine, or leptin levels. TBARS and FRAP also remained unaffected, despite previously reported anti-oxidative properties of DMG. Further, no significant impact on reproductive performance could be recorded. In conclusion, DMG supplementation significantly improved nutrient digestibility. Possible beneficial effects on energy metabolism and reproductive performance of sows should be tested when DMG is supplemented for a longer period of time or at a higher dose.

5 N,N-dimethylglycine in parturition feed

5.1 Introduction

The peripartal period is a critical period in commercial sow herds. During the last third of gestation, the sow's metabolic and hormonal state dramatically changes (Père et al., 2000; Weldon et al., 1994a). Sows can become catabolic during the last month of gestation (Close et al., 1985) which was evidenced by increased concentrations of non-esterified fatty acids (NEFA) and glycerol in plasma (Revell et al., 1998b). This is due to an increased fetal demand and can lead to reduced appetite (Weldon et al., 1994a) and in very rare cases even to porcine ketosis (Alsop et al., 1994). After day 85 of gestation, insulin sensitivity decreases (Père et al., 2000). Poor glucose tolerance during late gestation has also been reported (Weldon et al., 1994b) and has been related to an increased number of stillborn piglets (Kemp et al., 1996), postpartum hypophagia (Weldon et al., 1994a), hypogalactia and subsequently to increased pre-weaning piglet mortality (Ayoade, 2003).

Several studies indicate that both feed composition and amount of feed offered during late gestation are of major importance in preventing reproductive problems in the peripartal period and the first days of lactation. Overfeeding sows during late gestation and high back fat thickness at parturition predispose to several problems in the first days post-partum: low voluntary feed intake (Revell et al., 1998b), higher catabolic rate (Hulten et al., 1993), increased NEFA mobilization and decreased insulin secretion (Weldon et al., 1994a). In this regard, it is important to consider not only the amount of energy, but also the source from which the energy in derived. For instance, van der Peet-Schwering *et al.* (2004) reported a decreased glucose tolerance and an increased number of stillborn piglets when sows were fed extra energy from fat during late gestation. But, none of these problems were recorded when energy was supplied as starch, indicating the importance of feed components (van der Peet-Schwering et al., 2004).

Reproductive performance of sows is not only influenced by the general feed composition, but also feed additives can have an influence on production results. Choline is one of those supplements. It has been commonly used for many decades as an additive in sow feed and its beneficial effect on sow and piglet performance has been reported in several studies (Kornegay and Meacham,

1973). More recently, a study on supplementation of betaine, a metabolite of choline, reported significantly improved total tract digestibility of crude fat, crude ash and fibre in weaned piglets without affecting digestibility of crude protein and nitrogen-free extract (Eklund et al., 2006). A less known substance is N,N-dimethylglycine (DMG), which - just as choline and betaine - is related to glycine metabolism (Figure 5.1).
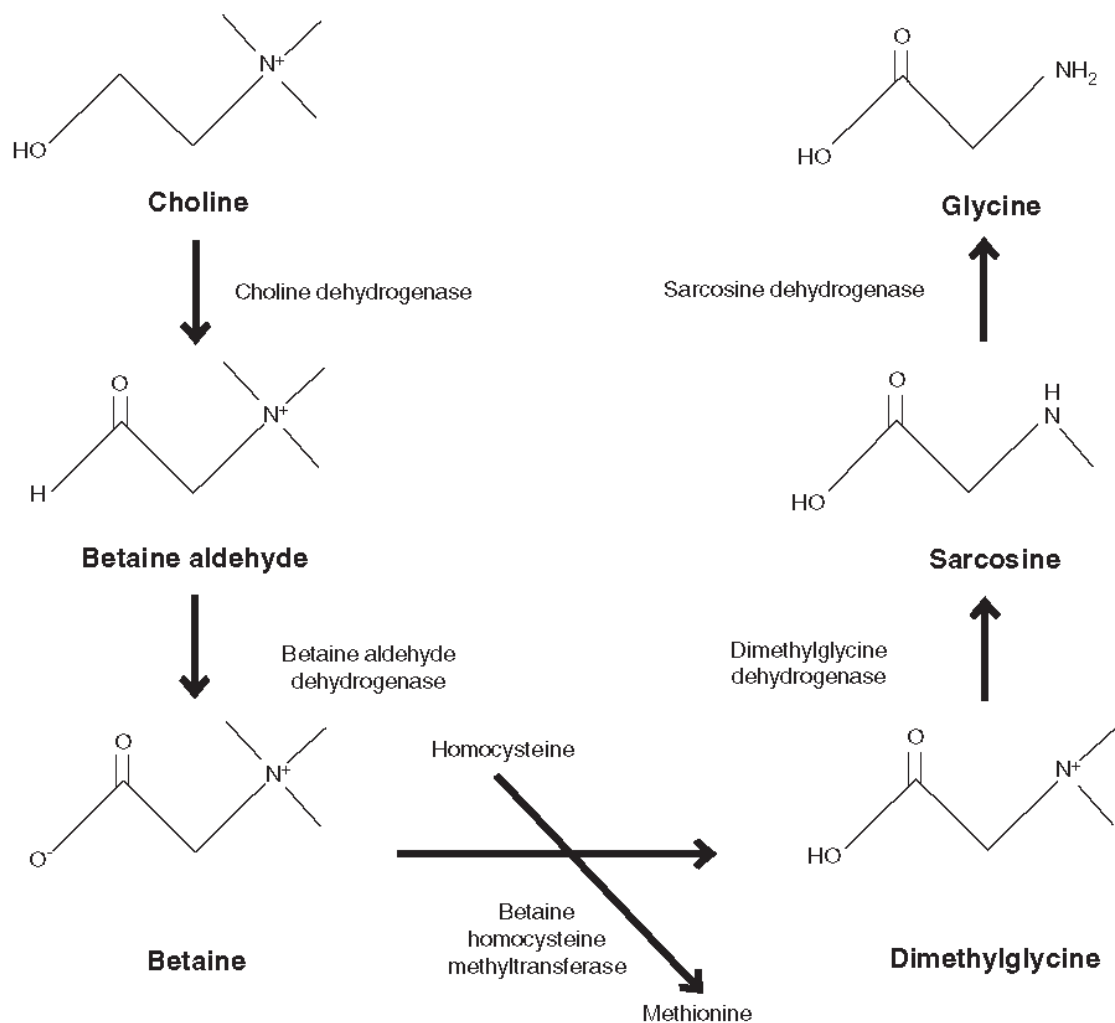

Figure 5.1 Overview of the choline pathway in which dimethylglycine (DMG) is an intermediate metabolite (own picture).

DMG can act as a methyl donor (Friesen et al., 2007) similar to betaine and additionally, it has anti-oxidative properties (Hariganesh and Prathiba, 2000). As methyl donation capacity (Jin et al., 2007) and anti-oxidative action (Lai, 2008) are both associated with increased insulin sensitivity, DMG can possibly influence insulin sensitivity. Other studies report that DMG, used as a dietary supplement, reduces blood lactate level and improves athletic performance in men (Tonda and Hart, 1992), horses (Greene et al., 1996) and dogs (Gannon and Kendall, 1982).

Only one study investigated the effect of DMG as a feed additive in livestock production, namely on broiler performance (Kalmar et al., 2010). In that study, an improved feed conversion rate of DMG-supplemented broilers was reported. Besides the application of DMG as a supplement for both humans and animals, DMG derived molecules are also used as surfactants in industrial applications (Guan and Tung, 1998) which indicates that DMG can possibly function as an emulsifying agent when added to the feed.

The current pilot study assessed the influence of DMG on insulin sensitivity, glucose and fat metabolism, nutrient digestibility and reproductive performance of sows in the peripartal period.

5.2 Materials and methods

The experiment was approved by the Ethical Committee of the Faculty of Veterinary Medicine, Ghent University Belgium (EC 2008/070) and by the Federal Public Service Health Food Chain Safety and Environment, Belgium.

5.2.1 Animals, experimental design and dietary treatment

The experiment was performed at the experimental farm of a feeding company (AVEVE N.V., Leuven, Belgium) during the months June, July and August 2008. In the herd a 3-week batch production system for sows was practiced. One group of the batch system, comprising 25 sows (Rattlerow Seghers hybrid) of different parities were included in the study. The sows were housed individually in mechanically ventilated stables with a 12 h light period from weaning until day 28 of gestation and they were fed a standard gestation diet (Table 5.1). Thereafter, the sows were moved to a group housing system with *ad libitum* access to a gestation feed, rich in soluble fibre for creating a feeling of satiety (Table 5.1). At day 105, the beginning of the experiment (day 1), the sows were removed from the gestation unit, body weight (BW) of each individual sow was recorded and they were transferred to the farrowing unit. They were housed individually in farrowing crates until weaning. Sows were randomly allocated into two treatment groups: a control group (n = 13, parity 3.8 ± 3.0) and a DMG group (n = 12, parity 3.8 ± 2.4).

Table 5.1 Feeding scheme and nutrient content of the different diets provided to the sows before, during and after the trial

Type of feed	Gestation (restricted)	Gestation (*ad libitum*)	Gestation (restricted)	Parturition (restricted)	Lactation (*ad libitum*)
Control				AIA[5]	
DMG			DMG[4]	AIA[5] & DMG[4]	
Feeding period[1]	Insemination → day 28	Day 29 → day 104	Day 105 → day 111	Day 112 → 3 days pp[6]	4 days pp[7] → weaning[8]
Dry matter[2]	884.9	907.8	884.9	902.9	911.3
Ash[2]	62.3	62.6	62.3	78.6	58.2
Crude protein[2]	135.3	135.5	135.3	148.6	160.6
Crude fat[2]	41.4	33.6	41.4	51.3	51.1
Crude fibre[2]	76.8	62.2	76.8	70.0	59.6
NE[3]	8.7	8.0	8.7	9.5	9.5

[1] Period of reproduction cycle during which the particular feed was given to the sows.
[2] Diet analyses (g/kg feed).
[3] Net energy (MJ/kg feed), calculated value using feed formulation software (Bestmix® Feed, Adifo N.V., Maldegem, Belgium).
[4] N,N-dimethylglycine (DMG), supplemented on top of feed at a ratio of 1 g/kg feed.
[5] Acid insoluble ash 1% added to the parturition feed.
[6] Three days post partum (pp).
[7] Four days post partum (pp).
[8] Weaning at day 26 ± 2 of lactation.

All sows were daily fed 3.5 kg of a standard gestation feed starting from day 105 of gestation until day 109 of gestation (day 5). On day 110 and 111 of gestation (day 6 and 7), all sows received 3.0 kg and 2.5 kg of the same standard gestation feed, respectively (Table 5.1). From day 112 of gestation (day 8) until three days after the calculated farrowing date (day 14), all sows received the same parturition feed containing 1% celite® (source of acid insoluble ash, AIA) as an external marker to measure apparent fecal digestibility (AFD, Table 5.1 and Table 5.2) (Sales and Janssens, 2003). The amount of parturition feed provided to each sow at day 112, 113 and 114 of gestation was 2.0 kg, 1.5 kg and 1.0 kg, respectively. One kg was given daily until parturition and from parturition onwards, the daily amount was increased again with 0.5 kg a day. On day 15 of the trial, all sows were switched to a standard lactation diet (Table 5.1) which was provided *ad libitum*. Feed samples of each diet were taken and analyzed according to the Association of Official Analytical Chemists (AOAC) methods (Table 5.1) (Thiex, 2002). For the parturition feed, also the amount of AIA was determined as described by Sales and Janssens (2003).

From day 1 (day 105 of gestation) until day 14 of the trial, all sows in the DMG group received 1 g DMG (Taminizer-D, supplied by Taminco N.V., Ghent, Belgium) per kg feed supplemented on top of their daily feed portion. The dose

of 1000 ppm was based on the supplementation levels recommended for choline (National Research Council, 1998).

During the entire experiment, all sows had free access to fresh drinking water (drinking nipple – flow 1.5-2 l/min).

Table 5.2 Composition of the parturition diet

Ingredient	%	Ingredient	%	Ingredient	%
Soybean meal	17.49	Wheat gluten feed	3.29	Sodium chloride	0.47
Barley	14.26	Maize	2.38	Linseed	0.42
Sugar beet pulp	14.26	Fish oil	1.98	Choline chloride	0.16
Wheat	12.55	Pork lard	1.98	Vitamin premix[3]	0.07
Wheat bran	9.50	Chalk	1.65	Methionine	0.05
Soy hulls	6.45	Mineral premix[2]	1.04	Luctarom S[4]	0.05
Molasses[1]	5.70	Celite	0.99	Trace elements [5]	0.02
Manioc	4.75	Soy oil	0.48	L-threonine	0.01

[1] Sugar beet molasses.
[2] Mineral premix: 4.8% mono iron sulphate, 0.5% copper sulphate, 85.3% magnesium phosphate, 6.8% monocalcium phosphate and 2.6% zinc sulphate.
[3] Vitamin premix: 6.33 g/kg vitamin A, 272.15 g/kg vitamin E, 0.063 g/kg vitamin D3, 5.02 g/kg vitamin K, 75.30 g/kg vitamin PP, 5.02 g/kg vitamin B1, 25.10 g/kg vitamin B2, 37.65 g/kg vitamin B3, 15.06 g/kg vitamin B6, 7.53 g/kg vitamin B9, 0.08 g/kg vitamin B12 and 0.75 g/kg vitamin H.
[4] Luctarom S is an industrial flavor, added to the feed to improve feed intake. The product has no nutritional value.
[5] Trace elements premix: 485.07 g manganese/kg premix, 1.02 g cobalt/kg premix, 12.03 g iodine/kg premix, 2.00 g selenium/kg premix

5.2.2 Blood sampling and oral glucose tolerance test

One day after parturition, a blood sample was taken and an oral glucose tolerance test (OGTT) was performed after an overnight fasting period of 12 h, as described by (Kemp et al., 1996) and (van der Peet-Schwering et al., 2004). For sows that started farrowing after 21:00, blood sampling and OGTT were performed two days after the actual farrowing date also after an overnight fasting period of 12 h.

At 07:00, a 30 ml blood sample was taken from the vena jugularis. Blood samples were divided into 4 subsamples: 9 ml on serum clot activator (fructosamine and insulin analyses), 9 ml on lithium heparin (leptin, triglycerides (TG), thiobarbituric acid reactive substances (TBARS), and ferric reducing ability of plasma (FRAP) analyses), 9 ml on K_3EDTA (non-esterified fatty acids (NEFA) analyses) and 3 ml on sodium fluoride/potassium oxalate (glucose analyses). The blood subsamples were stored on ice water (4 °C) for about 5 h. Subsequently, tubes were centrifuged at 3000 × g for 10 min and plasma and serum samples were stored frozen at -20 °C until analysis. After this blood sampling, the tail of each sow was washed with disinfectant soap. At 07:50, a blood sample from a small incision in the tail was taken and the glucose concentration in this blood

sample was measured using a glucose meter (Precision Xceed™, Abbott Diabetes Care, Louvain-la-Neuve, Belgium). This device has a coefficient of variation of 6.7% and accuracy is according to ISO 15197 standards for glucose meters (Scandinavian evaluation of laboratory equipment for primary health care (SKUP, 2006). Ten minutes later (08:00 h), sows were fed 3 g glucose per kg $BW^{0.75}$ and blood samples were taken at 11 time points (10, 20, 30, 40, 50, 60, 70, 80, 90, 105, and 120 min after glucose feeding) followed by glucose analysis as described above. Area under the curve (AUC) for glucose was calculated according to the trapezoidal method.

5.2.3 Colostrum and feces sampling

After the OGTT was completed, sows were fed their morning feed portion. Subsequently, a colostrum sample was taken from each sow after an intramuscular injection of 1.0 ml oxytocin. Finally, a fresh rectal feces sample of each sow was taken. Both colostrum and feces samples were stored frozen at - 20 °C until analysis.

5.2.4 Analyses of blood samples

Insulin was analyzed using an immunoradiometric assay kit (BioSource INS-IRMA Kit, BioSource Europe S.A., Nivelles, Belgium). Glucose and fructosamine were both determined spectrophotometrically (Roche/Hitachi Modular P Analyzer, Roche Diagnostics, Mannheim, Germany) by adding hexokinase and glucose-6-phosphate dehydrogenase (Gluco-quant, Roche Diagnostics, Mannheim, Germany) for glucose analysis and nitrotetrazolium-blue (Fructosamine, Roche Diagnostics, Mannheim, Germany) for fructosamine analyses. Triglycerides were also measured spectrophotometrically (Monarch Chemistry System, Instrumentation Laboratories, Zaventem, Belgium) using a commercial colorimetric diagnostic kit (IL Test kit No. 181610-60). Leptin in plasma samples was measured using a multispecies immunoradiometric assay kit (Multi-Species Leptin RIA Kit, Linco Research Inc., St Charles, Missouri, USA). Plasma NEFA concentrations were determined using a WAKO NEFA-HR(2) test (Wako Chemicals GmbH, Neuss, Germany), modified for use on the Monarch Chemistry System. Plasma lipid peroxidation, expressed as TBARS, was measured spectrophotometrically (Monarch Chemistry System, Instrumentation Laboratories, Zaventem, Belgium) as described in detail by Lin et al. (2004). Results are reported as the concentration of malondialdehyde (MDA) measured

per ml plasma after reaction. Similarly, total plasma anti-oxidative capacity of plasma, expressed as FRAP, was measured spectrophotometrically (Monarch Chemistry System, Instrumentation Laboratories, Zaventem, Belgium) as described by Benzie and Strain (1996). Results are reported as the concentration of Fe^{2+} measured per l of plasma after reaction.

5.2.5 Colostrum analyses

First, the colostrum samples were extracted with hexane to remove the fat fraction. The aqueous layer which contained the DMG was then separated from the hexane layer. Subsequently, diethylglycine sodium salt was added to the aqueous extract as an internal standard. After evaporation of the aqueous solution under a nitrogen stream, N,O-bis(trimethylsilyl)trifluoroacetamide and dimethylformamide were added to derivatise both the DMG and the internal standard. Finally, the sample was injected in a gas chromatograph equipped with a split injector and flame ionization detector. The results were calculated based on the internal standard.

5.2.6 Feces analyses

Feces samples of each sow were analyzed for AIA, dry matter, crude protein, crude ash, crude fat and crude fibre according to Thiex (2002) and NE of the feces was calculated using the Rostock formula. Using these results and the analyses of the parturition feed, AFD of these nutrients was calculated using the external marker method with AIA as external marker, according to the following formula:

$$AFD = [1 - (F / D \times D_{AIA} / F_{AIA})] \times 100$$

with F = % nutrient in the feces, D = % nutrient in the feed, F_{AIA} = % AIA in the feces and D_{AIA} = % AIA in the feed (Goddard and McLean, 2001).

5.2.7 Reproductive performance parameters

Following reproductive parameters of the sows were investigated: gestation length, number of total born, live born and still born piglets and the number of weaned piglets. The litter weight at birth and at weaning, and the mortality rate of piglets before weaning were also recorded.

5.2.8 Statistical analyses

The effect of DMG supplementation on glucose tolerance, blood parameters, AFD and reproduction parameters were analyzed using the Student *t*-test for independent samples after performing a Kolmogorov-Smirnov test to verify normality and a Levene's test to determine equality of variance. The DMG content in colostrum and the number of stillborn piglets were not normally distributed and therefore these data were analyzed using the non-parametric Mann-Whitney test. All statistical analyses were performed using SPSS 17.0 (SPSS Inc., Chicago, Illinois, USA), considering statistical significance when *P* < 0.05. All data are reported as mean ± standard deviation (SD).

5.3 Results

5.3.1 Oral glucose tolerance test

The OGTT was successfully performed in 11 sows of the control group and 10 sows of the DMG group. Four sows (two from the control and two from the DMG group) were excluded from the OGTT because their tail was too short to perform the protocol correctly. Basal glucose levels, measured 10 min before the sows were offered their individual glucose load, were similar for both treatments (4.2 ± 1.1 mmol/l and 4.1 ± 1.7 mmol/l for control and DMG group, respectively, *P* > 0.05). Glucose tolerance, represented as the AUC after the OGTT, was not significantly influenced by supplementation of DMG. The average AUC values were 612 ± 105 mmol × min/l for the control group and 650 ± 151 mmol × min/l for the DMG group. A maximal increase in blood glucose level of 2.4 ± 1.4 mmol/l above basal level was recorded for sows of the control group after 57 ± 35 min, which was not significantly different from the maximal increase observed in sows of the DMG group (2.7 ± 1.5 mmol/l after 54 ± 29 min). After 120 min, blood glucose levels had decreased again to 4.7 ± 0.8 mmol/l in the control group and to 5.2 ± 0.7 mmol/l in the DMG group (*P* > 0.05).

5.3.2 Blood parameters

The results of the blood parameters are given in Table 5.3. Supplementation of DMG had no significant influence on glucose, insulin or fructosamine (*P* > 0.05). Similarly, leptin concentration in blood samples was not significantly affected by dietary treatment (*P* > 0.05). Although no significant difference could be seen for NEFA (*P* > 0.05), TG tended to be lower (*P* = 0.067) for the control group (0.29

± 0.16 ng/mL) compared to the DMG group (0.47 ± 0.31 ng/mL). Neither TBARS nor FRAP were significantly affected by supplementation of DMG ($P > 0.05$).

Table 5.3 Results of the blood parameters of sows in the control group (n = 13) and the DMG group[1] (n = 12)

Parameter	Control group	DMG group	SEM	*P*-value
Glucose (mmol/l)	4.3	4.2	0.08	0.394
Insulin (mU/l)	18.3	21.2	2.6	0.597
Fructosamine (µmol/l)	247	241	3	0.397
Leptin (ng/ml)	1.9	1.8	0.1	0.874
TBARS (nmol/ml)[2]	1.3	1.5	0.1	0.318
FRAP (µmol/l)[3]	116	129	5	0.198
Triglycerides (mmol/l)	0.29	0.47	0.05	0.067
NEFA (mmol/l)[4]	0.63	0.77	0.06	0.291

[1] Sows of the DMG (N,N-dimethylglycine) group were supplemented with 1 g DMG/kg feed during 14 days.
[2] Thiobarbituric acid reactive substances (TBARS) expressed as the amount of malondialdehyde (MDA) measured in plasma.
[3] Ferric reducing ability of plasma (FRAP) expressed as the concentration of Fe^{2+} measured in plasma.
[4] Non-esterified fatty acids.

5.3.3 Apparent fecal digestibility of nutrients

Feces samples of 11 sows of the control group and 9 sows of the DMG group were analyzed. No rectal feces sample could be taken from two sows of the control group and three sows of the DMG group because these five sows were constipated. The AFD results are given in Table 5.4. AFD of crude fat, crude protein and of nitrogen-free extract were significantly improved in the DMG group compared to the control group ($P < 0.05$).

Table 5.4 Results (%) of the apparent fecal digestibility (AFD) of nutrients of sows of the control group (n = 11) and the DMG group[1] (n = 9)

Parameter[2]	Control group	DMG group	SEM	*P*-value
AFD crude fat	84	91	2	0.032
AFD crude protein	67	81	3	0.024
AFD NFE[3]	75	87	3	0.029
AFD NE[4]	67	82	4	0.027

[1] The DMG (N,N-dimethylglycine) group was supplemented with 1 g DMG/kg feed during 14 days.
[2] Feces samples were taken at day 1 post partum.
[3] Nitrogen free extract (NFE) calculated based on the complete proximate analyses of feed and feces samples (NFE = dry matter – crude fibre – crude fat – crude protein – crude ash).
[4] NE calculated using the Rostock formula
(NE = $10.8 \times$ crude protein + $36.1 \times$ crude fat + $6.3 \times$ crude fibre + $12.7 \times$ NFE)

5.3.4 Colostrum analyses

Colostrum samples of 13 sows of the control group and 11 sows of the DMG group were analyzed. From one sow of the DMG group, the amount of colostrum sampled was insufficient for analysis. The concentration of DMG in colostrum of both groups was low and not significantly different from each other (7 ± 7 µg/g and 11 ± 16 µg/g for control and DMG group respectively, $P > 0.05$).

5.3.5 Reproductive performance parameters

The reproductive performance parameters, shown in Table 5.5, were not significantly influenced by DMG supplementation ($P > 0.05$). However, the number of stillborn piglets was numerically lower in the DMG group (0.3 ± 0.6) than in the control group (0.9 ± 1.4, $P > 0.05$). In general, after a lactation period of 26 ± 2 days, an average number of 10.9 ± 1.9 piglets per litter were weaned with a total litter weight of 69 ± 10 kg.

Table 5.5 Results of reproductive performance parameters of sows of the control group (n = 13) and the DMG group[1] (n = 12)

Parameter	Control group	DMG group	SEM	P-value
Gestation length (days)	116.4	115.4	0.5	0.334
Number of total born piglets	13.9	13.6	0.5	0.759
Number of live born piglets	13.0	13.3	0.5	0.743
Number of stillborn piglets[2]	0.9	0.3	0.2	0.180
Litter weight at birth (kg)	19.2	20.5	0.5	0.259
Average piglet birth weight (kg)	1.5	1.6	0.05	0.469
Number of weaned piglets	10.9	10.8	0.4	0.908
Mortality during lactation (%)	13.8	18.5	3.2	0.466
Litter weight at weaning (kg)	68.8	69.0	2.1	0.966
Average piglet weaning weight (kg)	6.4	6.5	0.2	0.792

[1] The DMG (N,N-dimethylglycine) group was supplemented with 1 g DMG/kg feed during 14 days.
[2] In contrast to all the other parameters, the number of stillborn piglets was tested using a non-parametric test instead of a parametric test because data were not normally distributed.

5.4 Discussion

The results of the present study observed at day 1 postpartum indicated that preprandial blood glucose levels were almost twofold those recorded in studies testing glucose tolerance in a similar way at day 104 and 108 of gestation (Kemp et al., 1996; van der Peet-Schwering et al., 2004). Similarly, maximal increase in blood glucose level during the OGTT in the present study performed postpartum was higher and occurred later in time than in OGTT performed in late gestation (Kemp et al., 1996; van der Peet-Schwering et al., 2004). In line with the present study and the previously mentioned studies of Kemp et al. (1996) and van der Peet-Schwering et al. (2004), results of intravenous glucose tolerance tests (IVGTT) performed on sows at day 109 of gestation and at day 4 of lactation confirm that glucose half-life is shorter in late gestation than in early lactation (Quesnel et al., 2009). Different explanations can be given for the basal hyperglycemia and decreased glucose clearance rate observed in early lactation. Oliviero et al. (2008) reported elevated plasma cortisol levels for more than one day after parturition for sows housed in farrowing crates. These increased cortisol levels could explain the lowered insulin sensitivity of tissue at the

beginning of lactation (Kronfeld et al., 2005). Although cortisol was not measured in the present study, it could be a possible explanation for the observed lower glucose clearance in early lactation. Hence, interference of cortisol with glucose metabolism could mask a possible effect of DMG on the glucose metabolism in early lactation in sows. This could also explain the lack of influence of DMG supplementation on the preprandial glucose, insulin and fructosamine levels, all these parameters being related to the glucose metabolism.

Despite the anti-oxidative properties of DMG reported in rats (Hariganesh and Prathiba, 2000), no influence on the oxidative parameters TBARS and FRAP were reported in the present study. However, the doses used by Hariganesh and Prathiba (2000) were much higher than the dose used in the present trial (25 or 35 mg/kg body weight in rats, in contrast to at the highest approximately 15 mg/kg body weight for sows consuming the maximum of 3.5 kg feed/day in the present trial). Also the rats in this study suffered gastric ulceration resulting in a physiologically different status than sows on day one post partum.

Research on leptin in many different species, including humans, has revealed that this hormone is related to the energy metabolism (Barb et al., 2001; Budak et al., 2006). Moreover, levels of leptin in sows can vary in relation to the feeding level (Quesnel et al., 2009), feed composition, time after transition from one feed to another (Papadopoulos et al., 2009b) and body condition, mostly measured as back fat thickness (Prunier et al., 2001). In the present study, both feeding scheme and body condition of all sows were similar, resulting in similar fasting leptin levels at the first day of lactation for both treatment groups. Given the inducing role of leptin in insulin resistance, as reviewed by (Barb et al., 2001), these similar leptin levels likely reduced the chance of finding differences in glucose tolerance induced by different dietary treatments. Fasting NEFA levels were also similar between treatments, indicating a similar amount of energy mobilized from fat reserves in both treatment groups. The absence of an influence of DMG supplementation on leptin combined with lack of effect on fasting NEFA concentration, the equal feed intake and similar body condition of all sows indicate that the present DMG supplementation over a period of 10 days did not affect the lipid metabolism of sows in the peripartal period. Although no influence on lipid metabolism could be recorded, the slight increase in TG blood levels and the significant improvement in AFD of crude fat indicate that DMG

supplementation over a longer period of time or at a higher dose might have a beneficial influence on the lipid metabolism. Indeed, the results of AFD in the present study indicate that DMG may act as an external emulsifier in pig feed (Dierick and Decuypere, 2004; Wieland et al., 1993). The emulsifying effect of DMG incorporation in the sow's diet not only resulted in improvement of crude fat digestibility, but also improved digestibility of crude protein and nitrogen-free extract. These findings corroborate with the results of a previously published study on the addition of emulsifying agents to pig feed (Dierick and Decuypere, 2004). Studies on the effects of emulsification of fat sources in piglet feed indicate that the addition of emulsifiers to piglet feed improve nutrient digestibility, resulting in a reduction of feed costs (Jones et al., 1992; Odle et al., 1994). Seeing the limited number of studies published on the effects of addition of emulsifiers to the diets for sows, it would be worthwhile to do further research on the effect of DMG on nutrient digestibility.

Results of colostrum analyses in the present study indicate that the used technique is not sensitive enough to measure small amounts of DMG in colostrum samples. The low DMG levels detected in both groups indicate that little DMG is transferred into colostrum. This finding is in line with previous studies reporting that most DMG is eliminated from the organism by demethylation rather than by excretion (Lever et al., 2005) or in this case by secretion in colostrum.

No direct effect of DMG supplementation on reproductive performance parameters was noted, except for a numerical decrease in the number of stillborn piglets in litters of sows fed diets supplemented with DMG. The incidence of piglet mortality at or shortly after farrowing may be linked to an increased glucose intolerance of sows during late gestation (Kemp et al., 1996).

In conclusion, the present study demonstrated that short term supplementation of DMG had an emulsifying effect which resulted in an improved nutrient digestibility. However, neither a significant influence on glucose clearance one day after parturition nor improved reproductive performance parameters could be substantiated in the present trial. Further research, in which DMG is supplemented for a longer period and/or at a higher dose, is warranted to investigate possible beneficial effects of DMG on energy and lipid metabolism, health and performance of the sow and the piglets.

Acknowledgements

Chapter 6

Ad libitum feeding of peripartal sows

Ad libitum feeding of sows during the peripartal period affects evolution of body condition, reproduction results and metabolic profile

A. Cools, D. Maes, R. Decaluwé, J. Buyse, T.A.T.G. van Kempen, A. Liesegang, G.P.J. Janssens

To overcome negative energy balance during the peripartal period of sows, an ad libitum *feeding strategy (ADLIB) as alternative for commonly used restricted feeding (STANDARD) was evaluated. Plasma metabolites and thyroid hormones, evolution of back fat thickness (BF), reproductive traits, and piglet performance were monitored. Voluntary feed intake of ADLIB sows dropped at farrowing but was still more than twice the amount of what was offered to STANDARD sows. Consequently, ADLIB sows lost less BF than STANDARD sows (P = 0.041). Additionally, BF change was affected by body condition. LEAN sows (BF < 18 mm on day 105 of gestation) lost less BF than MODERATE sows (18 mm < BF < 22 mm) which lost less BF than FAT sows (BF > 22 mm) (P < 0.001). Except for a lower percentage of stillborn piglets for MODERATE sows (P = 0.044), reproduction results were not affected. Piglet weaning weight of ADLIB-FAT and STANDARD-MODERATE sows was lower than that of ADLIB-LEAN sows (P = 0.005). Regardless of body condition, all metabolites and thyroid hormones measured showed a time dependent profile (P < 0.001). On day 112 of gestation higher levels of creatinine (P = 0.004), non-esterified fatty acids (P = 0.039), and serum crosslaps (P = 0.016) for STANDARD sows were observed. Triglycerides were higher for FAT sows (P < 0.001), and time×feeding strategy (P = 0.013) and time×condition (P = 0.012) interactions were observed. Although* ad libitum *feeding during the peripartal period only resulted in less mobilization of muscle, fat, and bone reserves on day 112 of gestation, results of BF evolution and piglet weaning weight indicated that* ad libitum *feeding is beneficial for sow performance provided that BF is below 22 mm.*

6 *Ad libitum* feeding of peripartal sows

6.1 Introduction

Nutrient demands of sows increase exponentially towards the end of gestation (Noblet et al., 1985) resulting in a catabolic metabolism (Le Cozler et al., 1999). Research has pointed out that increasing energy intake during late gestation results in higher milk production (Coffey et al., 1987), higher milk fat content (Boyd et al., 1978), and higher preweaning survival (Cieslak et al., 1983; Seerley et al., 1974). Additional protein intake during late gestation results in increased litter birth weight (Yang et al., 2009) and higher milk protein levels (Al-Matubsi et al., 1998). After farrowing, vast amounts of nutrients and energy are needed for milk production. During lactation additional energy and protein intake resulted in increased milk production (Dourmad et al., 1998), increased litter weight gain (Dourmad et al., 1998; Nelssen et al., 1985a), reduced mobilization of sows' body reserves (McNamara and Pettigrew, 2002), shorter weaning to oestrus interval (Koketsu et al., 1998; Sterning et al., 1990), and improved reproductive performance during the subsequent cycle (Cromwell et al., 1989; Dourmad et al., 1994; Koketsu et al., 1997). However, a reduction in feed intake during the peripartal period is often recommended to prevent a drop in lactational feed intake (Koketsu et al., 1996; Kruse et al., 2011). Moreover, sows consumed more feed than needed during gestation (Eissen et al., 2000) resulting in increased fat reserves which decreased lactational feed intake (Dourmad, 1991; Prunier et al., 2001; Weldon et al., 1994a). Others identified high feed intake postpartum as a risk factor for the occurrence of peripartal hypogalactic syndrome (Papadopoulos et al., 2010). Yet, only few studies investigated the effect of *ad libitum* feeding introduced at the beginning of the peripartal period. *Ad libitum* feeding around farrowing seems an unknown practice, although there is no sound body of evidence why this would be counterproductive, and much seems based on tradition. A study of Neil (1996) revealed that the earlier *ad libitum* feeding was introduced during the peripartal period, the less body reserves were mobilized. However, in that study the body condition of sows, which can have an influence on lactational voluntary feed intake (Revell et al., 1998b), was not taken into account. The present study, therefore, investigated the effect of body condition and *ad libitum* feeding during

the peripartal period, in comparison to a commonly applied restricted feeding strategy, on evolution of back fat and reproductive performance. Fat metabolism was investigated by measuring non-esterified fatty acids (NEFA) and triglycerides (TG) (Hulten et al., 1993), catabolism of lean tissue by measuring creatinine (CREA) (Yang et al., 2009), bone metabolism by measuring osteocalcin (OC) and serum crosslaps (CTX) (Lauridsen et al., 2010; Liesegang et al., 2005), and metabolic rate was assessed by measuring thyroid hormones (3,3′,5-triiodothyronine (T_3) and thyroxine (T_4)) (Samanc et al., 2010). We hypothesised that *ad libitum* feeding of sows around farrowing is a strategy for limiting negative energy balance and avoid distinct energy balance changes, hence promoting reproductive performance.

6.2 Materials and methods

The trial was performed in accordance with the Polish legislation on animal experiments and all animals were housed in accordance with EU legislation on animal husbandry. Animal health throughout the entire experiment was under veterinary supervision and no serious health problems were observed.

The experiment was performed simultaneous with the experiment described in chapter 4.

6.2.1 Animals, experimental design, and dietary treatment

At the experimental farm of Provimi Poland Sp.z.o.o. (Bieganów, Cybinka, Poland) a total of 112 sows (PenArLan Naïma hybrid) with parity two to seven were selected from two subsequent groups of the one-week batch production system (54 sows from the first and 58 sows from the second group). From insemination until day 35 of gestation, all sows were housed individually in the insemination unit. At day 36 post insemination, sows were moved to the gestation unit where they were housed in groups of ten until day 105 of gestation. From insemination until day 105 of gestation, all sows were fed a standard gestation pellet (898.0 g/kg dry matter, 133.1 g/kg crude protein, 14.6 g/kg crude fat, 49.6 g/kg crude fibre, 50.4 g/kg crude ash, all on as-fed basis) following a restricted feeding scheme (Figure 6.1). At day 105 of gestation, after consumption of their last portion of gestation feed, sows were transferred to the farrowing unit where sows were housed in individual pens with a farrowing crate for the sow and a solid floor piglet nest heated by an infrared heating lamp.

During transfer from the gestation to the farrowing unit body weight of each sows was assessed together with measurement of back fat thickness at the P2 position (Maes et al., 2004) using a Renco Lean-Meater (S.E.C. Repro, Ange-Gardien-de-Rouville, Québec, Canada). Based on equal distribution of both parity and back fat thickness, sows were randomly assigned to one of two treatment groups. Starting from day 106 of gestation onwards, all sows were fed three portions (6:00, 12:00, and 16:00) daily of the same lactation pellet (891.9 g/kg dry matter, 160.8 g/kg crude protein, 34.9 g/kg crude fat, 54.6 g/kg crude fibre, 48.9 g/kg crude ash, 9.24 MJ/kg calculated net energy, all on as-fed basis) until weaning. A control group (STANDARD n = 57) was fed the lactation feed during the peripartal period (d 106 of gestation until day 7 of lactation) following a decreasing-increasing feeding scheme (Figure 6.1). The treatment group (ADLIB n = 55) was fed the lactation feed *ad libitum* (with a maximum of 9 kg/day) during the peripartal period. Sows of the ADLIB group were offered three times daily a feed portion of 3 kg if the previous portion was consumed. Based on the number of consumed portions and the leftovers of the previous portion, daily feed intake was calculated. From day 8 of lactation onwards, all sows were fed *ad libitum* until weaning. During the entire period in the farrowing unit, all sows had *ad libitum* access to fresh drinking water.
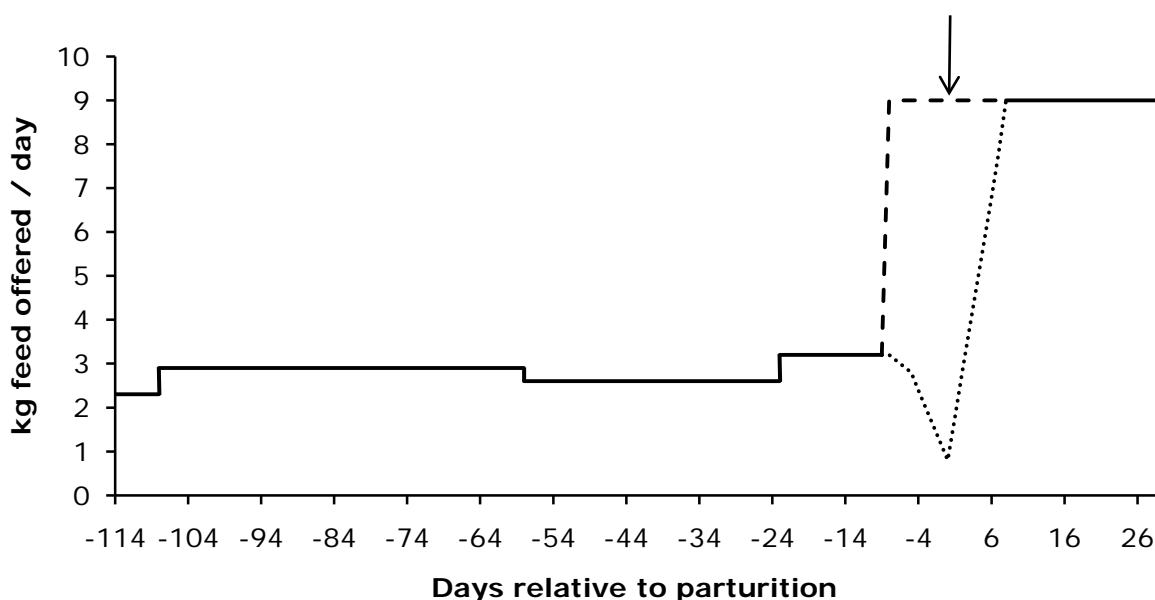
Figure 6.1 Feeding scheme of sows. During the gestation period all sows followed the same feeding scheme untill day 105 (———). From day 106 sows were either fed *ad libitum* (- - - - -) or following a restricted feeding scheme (· · · ·) until day 7 of lactation. From day 8 of lactation until weaning, all sows were fed *ad libitum*. The vertical arrow on the graph indicates the day of farrowing.

Based on back fat thickness (P2 position) measured at day 105 of gestation, sows were assigned to one out of three body condition groups. When back fat thickness was lower than 18 mm, sows were classified as LEAN (L, ADLIB-L, n = 19; STANDARD–L, n = 23); when back fat was more than 22 mm, sows were classified as FAT (F, ADLIB-F, n = 14; STANDARD-F, n = 16); and when sows had back fat levels between 18 and 22 mm, they were classified as MODERATE (M, ADLIB-M, n = 22; STANDARD-M, n = 18).

6.2.2 Reproductive performance

Reproductive parameters (gestation length, total born, live born, and stillborn) for each individual sow were recorded. For sows included in the trial, farrowing was not induced and farrowing duration of each sow (time between birth of the first piglet and expulsion of all placentae) was monitored. Within 24 h postpartum, litters were standardized (10 ± 1 piglet per litter) by cross-fostering piglets in order to become a comparable metabolic demand for each sow.

6.2.3 Sow body condition and piglet performance

Back fat thickness was measured at the P2 position, both at the left and right of the spine and average back fat thickness per sow was calculated. Next to the initial measurement at day 105 of gestation, this measurement was repeated at day 107, 109, 111, and 113 of gestation and at day 1, 3, 5, 7, 14, 21, and 29 of lactation. Body weight of sows was determined upon entering the farrowing unit at day 105 of gestation and at weaning.
Litter size and weight were determined between 24 and 36 h after farrowing and again at day 3, 5, 7, 14, 21, and 29 of lactation.

6.2.4 Blood samplings

At day 107, 109, and 112 of gestation and at day 1, 3, and 5 of lactation, 32 sows of the STANDARD group (STANDARD-L, n = 10; STANDARD-M, n = 13; STANDARD-F, n = 8) and 31 sows of the ADLIB group (ADLIB-L, n = 9; ADLIB-M, n = 13; ADLIB-F, n = 10) were selected for blood sampling. A 10 mL blood sample was taken from the vena jugularis between 8:00 and 9:00 (two hours after the start of the morning meal). Immediately after sampling, each blood sample was divided into three subsamples: 2 ml in tubes with serum cloth activator (for analysis of CTX and OC), 4 ml in tubes with lithium heparin (for analysis T_3 and T_4) and 4 ml in tubes with K_3EDTA (for analysis CREA, TG, and

NEFA). The subsamples were stored at 4°C until centrifugation. Subsequently, tubes were centrifuged (1000 × g for 10 minutes) and plasma and serum samples were stored frozen at -20°C until further analysis.

6.2.5 Blood analyses

Osteocalcin was measured using a commercial enzyme-linked immuno sorbent assay (ELISA) kit for measuring intact human osteocalcin (MicroVueOsteocalcin, Quidel Corporation, San Diego, USA). Serum crosslaps, which is the bone-derived degradation product of type I collagen C-telopeptides (CTX), was used as marker for bone degradation. This parameter was analyzed with a commercial one step ELISA kit for human CTX (Serum CrossLaps ELISA, Nordic Bioscience Diagnostics A/S, Herlev, Denmark). Both analytical kits were previously validated for analyses of porcine blood samples (Lauridsen et al., 2010). Creatinine and TG were both determined spectrophotometrically (Ultrospec III Pharmacia LKB Ltd., Cambridge, United Kingdom) by adding picric acid and sodium hydroxide (Randox Crea, Randox Laboratories Ltd., Crumlin, United Kingdom) for creatinine analysis and lipases, glycerol-kinase, glycerol-3-phosphate oxidase and peroxidase (Randox Trigs, Randox Laboratories Ltd., Crumlin, United Kingdom) for TG analysis. Non-esterified fatty acids were also measured spectrophotometrically (Victor 3 Multilabel Plate Reader, PerkinElmer Inc., Waltham, Massachusetts, USA) by adding acyl-CoA synthetase, acyl-CoA oxidase and peroxidase (Wako NEFA-HR, Wako Chemicals GmbH, Neuss, Germany). Both thyroid hormones T_3 and T_4 were determined using a specific radio-immunoassay (RIA) as described by Darras et al. (1992). Samples were all analyzed in one run to avoid interassay variations.

6.2.6 Statistical analyses

All data were entered into Microsoft Office Excel 2007 (Microsoft Corporation, Redmond, Washington, USA) and statistical analyses were performed using SPSS Statistics 21.0 (SPSS, Chicago, Illinois, USA). Prior to statistical analyses, homogeneity of variance and normality of data were tested by means of Levene's test and Kolmogorov-Smirnoff test, respectively. Number and percentage of stillborn piglets, and number of mummies were not normally distributed and, therefore, these parameters were subjected to a none-parameteric Kruskal-Wallis test for investigating the effect of either feeding strategy or body condition and significant differences were detected using a Dunn's test. Parameters with

successive measurements over time were subjected to repeated measurements analyses of variance (ANOVA) with time as within-subject factor and both feeding strategy and body condition as between-subject factor. For evolution of back fat thickness, the first measurement was included as covariable in the model. When not significant, the interaction term feeding strategy × body condition was removed from the model. Possible differences between different time points were assessed using the least significant differences (LSD) test with Bonferroni correction. Possible differences for between-subject factors were discriminated by means of a post-hoc Tukey test. To investigate differences in metabolic parameters at individual days, data at each time point separately were subjected to a univariate ANOVA with feeding strategy and body condition as fixed factors. Similarly, parameters that were measured only once were analyzed using a univariate ANOVA with both feeding strategy and body condition as fixed factors. Again, when not significant, the interaction term feeding strategy × body condition was removed from the model and a post-hoc Tukey test was performed when appropriate. Data are reported as mean ± standard deviation (SD) except when reported otherwise, and statistical significance was considered when $P <$ 0.05.

6.3 Results

Voluntary feed intake (VFI) of the ADLIB group changed over time during the entire period in the farrowing room ($P <$ 0.001, Figure 6.2) but was not affected by body condition. Only considering the peripartal period, the change of VFI over time was situated in the period around farrowing ($P <$ 0.001, Table 6.1) and not during lactation weeks 2 to 4 ($P >$ 0.05). On the day of farrowing feed intake averaged 4.24 ± 2.11 kg and was not affected by body condition of sows. No individual cases of peripartal hypophagia were observed amongst sows included in the trial. Total loss of body weight between day 105 of gestation and weaning was not affected by feeding strategy or body condition (Table 6.2). In contrast, loss of back fat during the same period of time was affected by feeding strategy ($P =$ 0.041) and body condition ($P <$ 0.001). Back fat thickness changed during the period in the farrowing room ($P =$ 0.001), with higher back fat levels for the ADLIB group ($P =$ 0.015) and a time × condition interaction ($P =$ 0.004). Taking a closer look at the peripartal and the lactation period separately, back fat thickness in both periods changed over time ($P =$ 0.006 peripartal and $P =$ 0.005

lactational). During the peripartal period back fat levels of ADLIB sows were higher (P = 0.031) whereas lactation tended to show a feeding strategy × time interaction (P = 0.054) resulting in a faster decline of back fat for ADLIB sows regardless of their condition (Figure 6.3).

Table 6.1 Voluntary feed intake (VFI) of sows fed *ad libitum* (ADLIB) from day 106 of gestation until weaning. Based upon back fat thickness (BF) at day 105 of gestation at the P2 position, sows were either classified as LEAN (BF < 18 mm), MODERATE (18 mm ≤ BF ≤ 22 mm), or FAT (BF > 22 mm)

| | ADLIB | | | | |
	LEAN	MODERATE	FAT	SEM	C[1]
Total: parturition -5 d until parturition + 25 d					
VFI (kg)	248.1	245.3	249.4	2.4	NS
VFI/d (kg/d)	8.0	7.9	8.0	0.08	NS
Peripartal: parturition − 5 d until parturition + 7 d					
VFI (kg)	96.3	92.6	96.1	1.4	NS
VFI/d (kg/d)	7.4	7.1	7.4	0.11	NS
Lactation: parturition + 8 d until parturition + 25 d					
VFI (kg)	151.9	152.7	153.2	1.3	NS
VFI/d (kg/d)	8.4	8.5	8.5	0.07	NS

[1] *P*-value of effect of body condition (C) on VFI, when not significant this was indicated with NS
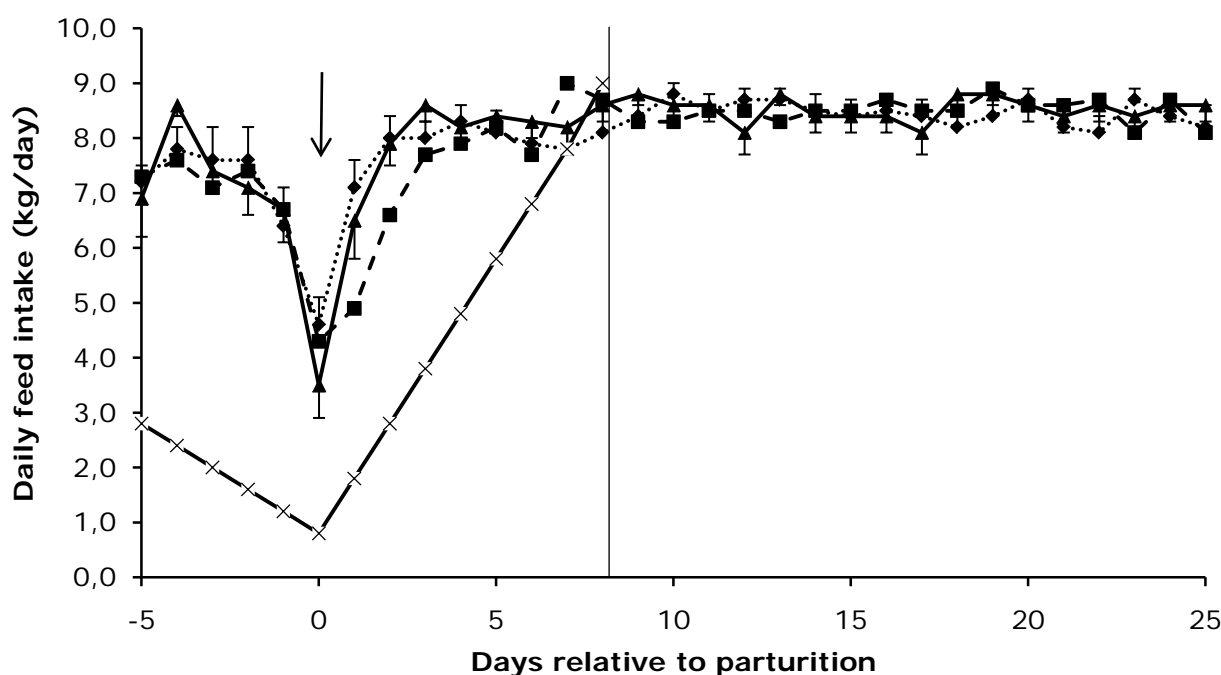
Figure 6.2 Voluntary feed intake (VFI) of sows fed *ad libitum* during the peripartal period (mean ± SEM). Based upon back fat thickness on day 105 of gestation sows were classified in one out of three body condition groups (C): LEAN (BF < 18 mm, ·· ♦ ··), MODERATE (18 mm < BF < 22 mm, - - - ■ - - -), or FAT (BF > 22 mm, —▲—). The standard applied restricted feeding scheme is presented on the graph (—×—) in order to allow comparison with the VFI of *ad libitum* fed sows. The vertical arrow on the graph indicates the moment of farrowing, the vertical line on the graph seperates the peripartal period from the rest of lactation.

Table 6.2 Overview of body condition parameters of sows and performance of their piglets. Sows were fed following a standard restricted feeding scheme (STANDARD) or were fed *ad libitum* (ADLIB) during the peripartal period. Based on back fat thickness at the P2 position at the start of the peripartal period, sows were classified as LEAN (BF < 18 mm), MODERATE (18 mm $\leq$ BF $\leq$ 22 mm), or FAT (BF > 22 mm).

	STANDARD			ADLIB			SEM	F × C[1]	F[1]	C[1]
	LEAN	MODERATE	FAT	LEAN	MODERATE	FAT				
Sows body condition[2]										
Weight changes[3]	-32.6	-48.4	-46.0	-47.7	-39.9	-38.1	2.8	NS	NS	NS
Back fat changes total[4]	-1.5^{Aa}	-3.4^{Ab}	-5.4^{Ac}	-0.9^{Ba}	-2.6^{Bb}	-3.5^{Bc}	0.3	NS	0.041	< 0.001
Back fat changes peripartal[5]	0.4^{Aa}	-0.9^{Ab}	-0.9^{Ab}	1.7^{Ba}	0.5^{Bb}	-0.8^{Bb}	0.2	NS	0.005	< 0.001
Piglet performance[2]										
Litter weight day 1[6]	17.47	17.66	17.12	17.00	16.44	16.31	0.29	NS	NS	NS
Litter weaning weight[6]	80.21	80.25	86.97	84.58	86.25	78.75	1.47	NS	NS	NS
Average piglet weight day 1[7]	1.54	1.60	1.53	1.67	1.51	1.58	0.03	NS	NS	NS
Average piglet weaning weight[7]	7.97^{xy}	7.53^{x}	8.42^{xy}	8.91^{y}	8.66^{xy}	7.7^{x}	0.12	0.005	(0.056)	NS
Average piglet growth[8]	6.52^{xy}	5.99^{x}	6.86^{xy}	6.98^{y}	6.97^{y}	6.10^{xy}	0.14	0.034	NS	NS
Average piglet growth week 1[9]	1.36	1.34	1.79	1.42	1.52	1.39	0.06	NS	NS	NS

[1] Interaction feeding strategy × body condition (F × C), feeding strategy (F), body condition (C): *P*–value is reported unless not significant (NS), when only a trend was observed (*P* < 0.10), *P* – value was enclosed by brackets.

[2] Results within one row with different capital letters (A - B) represent differences between feeding strategy, differences caused by body condition were identified by small letters (a – c), and differences caused by feeding strategy × body condition interaction were indicated by small letters (x - y).

[3] Weight change (kg) calculated between day 105 of gestation and weaning, negative values represent loss of weight.

[4] Back fat change (mm) at the P2 position between day 105 of gestation and weaning, negative values represent back fat loss.

[5] Back fat change (mm) at the P2 position between day 105 of gestation and day 7 of lactation, negative values represent back fat loss.

[6] Weight of total litter (kg).

[7] Piglet weight calculated by dividing litter weight by the number of piglets (kg).

[8] Piglet growth between day 1 of lactation and weaning (kg) calculated on average piglet weight.

[9] Piglet growth between day 1 and day 7 of lactation (kg) calculated on average piglet weight.

Table 6.3 Reproduction results of sows either fed restricted (STANDARD) or *ad libitum* (ADLIB) during the peripartal period. Based upon back fat thickness at the P2 position sows were classified as LEAN (BF < 18 mm), MODERATE (18 mm < BF < 22 mm), or FAT (BF > 22 mm)

	STANDARD			ADLIB			SEM	F[1]	C[1]
	LEAN	MODERATE	FAT	LEAN	MODERATE	FAT			
Gestation length (days)[2]	112.7	113.7	112.9	113.3	113.5	113.5	0.1	NS	NS
Farrowing duration (min)	336	342	385	344	325	303	10	NS	NS
Farrowing duration/piglet	30	32	32	33	33	29	1	NS	NS
Total born	11.7^A	10.6^A	12.2^A	10.4^B	10.6^B	10.6^B	0.2	0.037	NS
Live born	11.2^A	10.4^A	11.6^A	9.9^B	10.4^B	10.4^B	0.2	0.047	NS
% live born	95.6	98.8	95.2	94.4	98.2	97.5	0.6	NS	(0.052)
Stillborn	0.5	0.2	0.6	0.5	0.2	0.3	0.07	NS	(0.051)
% stillborn	4.4^a	1.2^b	4.8^a	5.6^a	1.8^b	2.5^a	0.6	NS	0.044
Mummies	0.5	0.3	0.2	0.3	0.1	0.2	0.06	NS	NS

[1] Feeding strategy (F), body condition (C): P –value is reported unless not significant (NS), when only a trend was observed, P – value was enclosed by brackets.
[2] Results within one row with different capital letters (A - B) represent differences between feeding strategy, differences caused by body condition were identified by small letters (a – c).
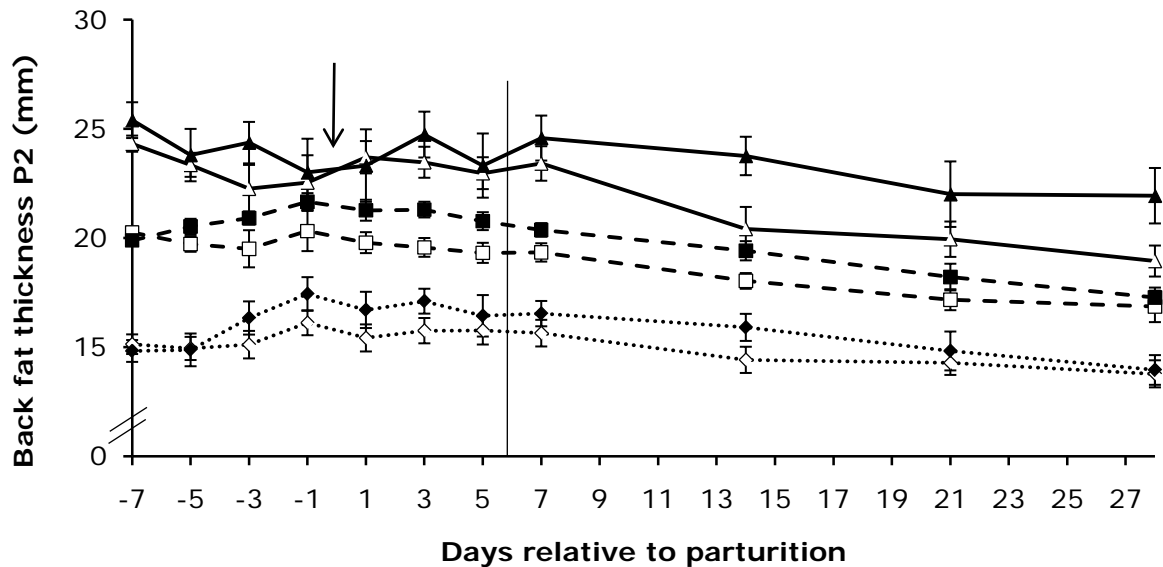
Figure 6.3 Evolution of back fat thickness (mean ± SEM) measured at the P2 position. During the peripartal period sows were fed following one of two feeding strategies (F): restricted (STANDARD, white symbols) or *ad libitum* (ADLIB, black symbols). Based upon back fat thickness on d 105 of gestation sows were classefied in one out of three body condition groups (C): LEAN (BF < 18 mm, STANDARD ··· ◇ ···, ADLIB ··· ♦ ···), MODERATE (18 mm ≤ BF ≤ 22 mm, STANDARD - - -□- - -, ADLIB - - - ■ - - -), or FAT (BF > 22 mm, STANDARD ——Δ——, ADLIB ——▲——). The vertical arrow on the graph indicates the day of farrowing, the vertical line on the graph seperates the peripartal period from the rest of lactation.

As could be expected, both average piglet weight and total litter weight increased significantly over time ($P < 0.001$, data not shown). Total litter weight and average piglet weight at day 1, however, did not differ significantly between feeding strategies or condition groups (Table 6.2). At weaning a feeding strategy × condition interaction ($P = 0.005$) was recorded with significantly heavier piglets of ADLIB-L sows compared to STANDARD-M and ADLIB-F sows. However, growth during the first week of lactation was similar for all piglets (Table 6.2).

An overview of the reproduction results is presented in Table 6.3. Both total born ($P = 0.037$) and live born piglets ($P = 0.047$) were higher for sows of the STANDARD group, regardless of body condition. However, when considering percentage of live born, M sows tended to have a higher percentage of live born piglets than the others ($P = 0.052$). Consequently, percentage of stillborn piglets was lower for the M sows ($P = 0.044$).

All measured metabolites showed a time-dependent profile throughout the peripartal period ($P < 0.001$, Figure 6.4). Except for TG, body condition did not affect the peripartal profile of the measured metabolites.
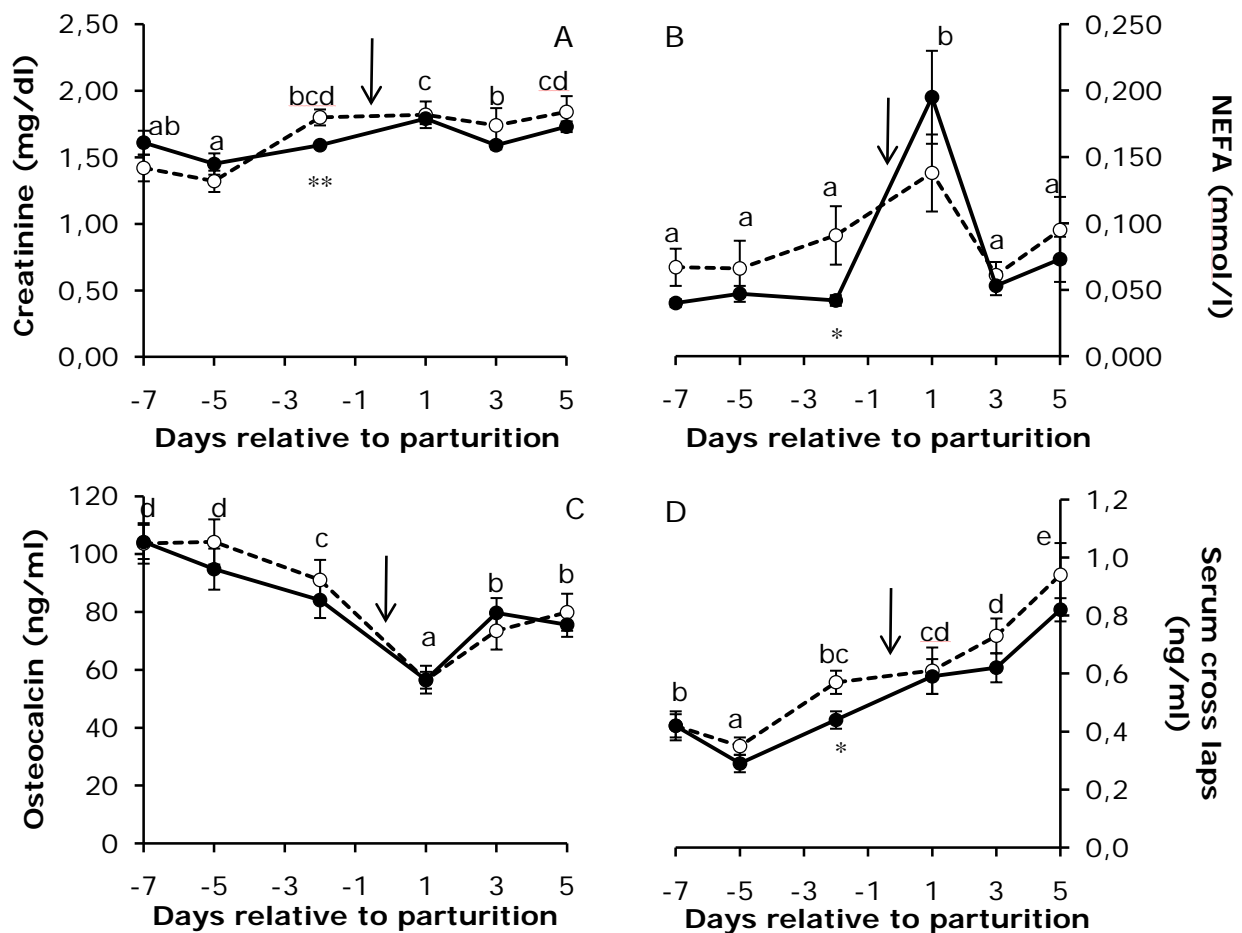
Figure 6.4 Peripartal profiles (mean ± SEM) of creatinine (A), NEFA (B), osteocalcine (C), and serum crosslaps (D) of sows either fed restricted (- - O - -) or *ad libitum* (—•—) during this period. The day of farrowing is indicated with a vertical arrow. Significant differences between feeding strategies at a certain time point are marked by * (*P* < 0.05) or ** (*P* < 0.01), differences between time points are indicated with different letters.

To simplify interpretation of graphs, the effect of body condition was omitted for all metabolites, except for the TG profile as there was an interaction between feeding strategy and body condition. Only on day 112 of gestation an effect of feeding strategy on CREA (*P* = 0.004), NEFA (*P* = 0.039), and CTX (*P* = 0.016) without influence of body condition, could be detected. Fat sows had higher levels of TG throughout the entire peripartal period (*P* < 0.001). Also interactions between time × feeding strategy (*P* = 0.013) and time × condition (*P* = 0.012) could be observed (Figure 6.5). Furthermore, TG tended to show a time × feeding strategy × condition interaction (*P* = 0.057) with higher post-prandial TG levels for ADLIB-F sows compared to STANDARD-L, ADLIB-L, STANDARD-M, and ADLIB-M sows. Similar to the measured metabolites also thyroid hormones showed a time-dependent profile (*P* < 0.001) regardless of body condition or applied feeding strategy (Figure 6.6).
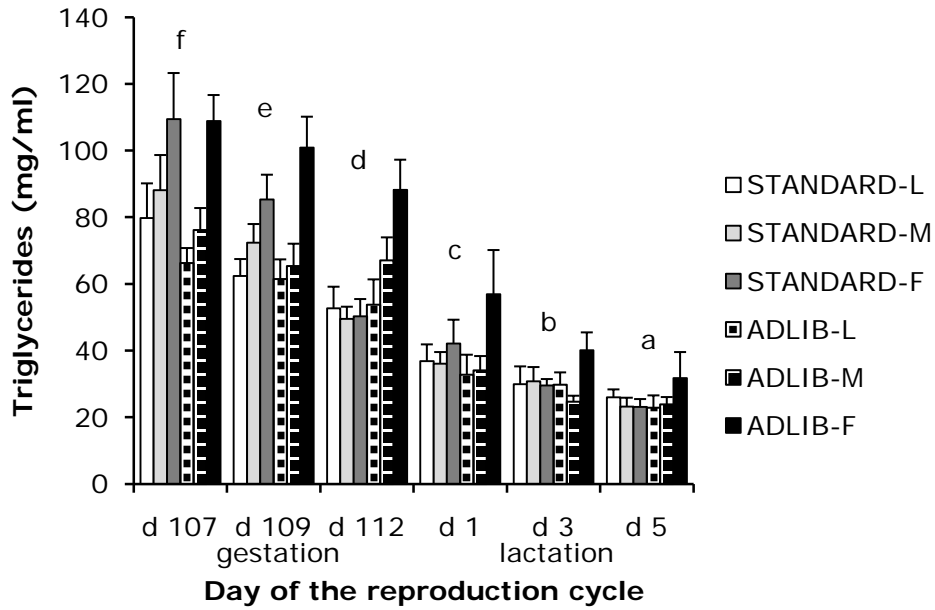
Figure 6.5 Peripartal profile of triglycerides (mean ± SEM) during the peripartal period of sows either fed *ad libitum* (ADLIB) or restricted (STANDARD). Based upon back fat thickness at the P2 position on day 105 of gestation, sows were classified as LEAN (BF < 18 mm), MODERATE (18 mm < BF < 22 mm), or FAT (BF > 22 mm).
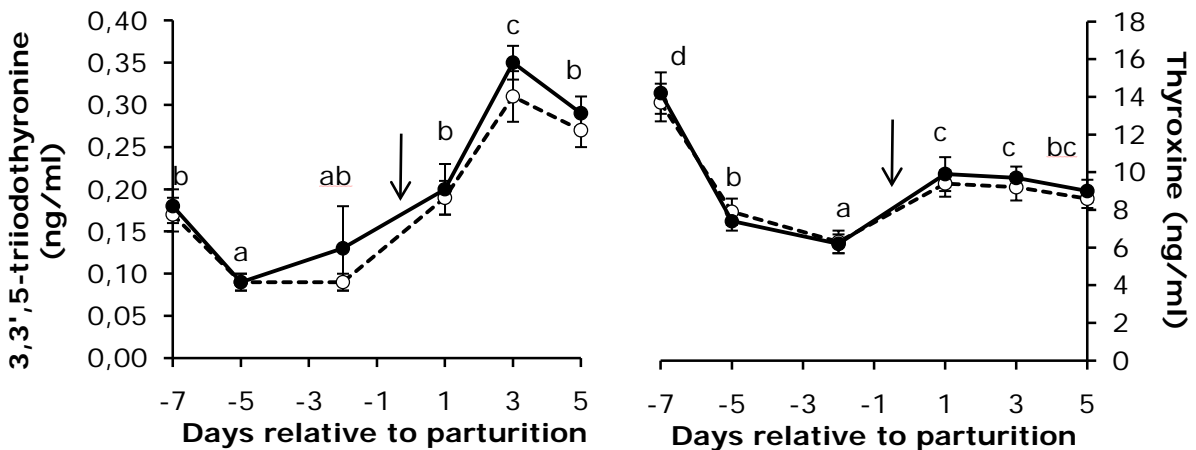
Figure 6.6 Peripartal profiles of thyroid hormones (mean ± SEM) of sows either fed restricted (- - - O - - -) or *ad libitum* (——•——) during this period. The day of farrowing is indicated with a vertical arrow. Significant differences between time points are indicated with different letters (a – d).

6.4 Discussion

The present study demonstrated that peripartal sows, when fed *ad libitum*, voluntarily lowered their feed intake around farrowing without suffering from any hypophagia problems afterwards. These findings corroborate earlier research of Neil (1996), who also reported a decreased feed intake the day of farrowing when *ad libitum* feeding was introduced four days before farrowing. Surprisingly, even though sows of the STANDARD group were offered only 46.9% (44.4 kg) of

the VFI of the ADLIB group (94.8 ± 10.4 kg) during the peripartal period and lactational feed intake was not different between both groups, no differences in weight loss at weaning could be detected. Nevertheless, back fat loss was reduced by increased peripartal feed intake, in line with previous findings (Guedes and Nogueira, 2001; Koketsu, 1999; Neil, 1996). The lack of correlation between loss of body weight and loss of back fat was also reported previously (Koketsu, 1999; Neil, 1996; Sterning et al., 1990). Whereas back fat loss only represents mobilization of fat reserves, weight loss can also imply mobilization of lean tissue. Voluntary feed intake throughout lactation was, in contrast to what is reported in most other studies (reviewed by Eissen et al., 2000), not affected by condition shortly before farrowing. We assume that the composition of the present lactation diet might have prevented a potential effect of body condition on VFI. In contrast to several other studies, the lactation diet used in the present study was high in carbohydrates (59 % nitrogen free extract) and low in fat (3.5 % crude fat). When sows shift from carbohydrate to fat metabolism ketosis may develop (Alsop et al., 1994). Moreover, in sows primary ketosis can be induced using lactation diets high in fat (10% crude fat) (Theil et al., 2012). So, therefore, the use of a high starch-low fat lactation diet might have been preventive for ketosis. Even though fat sows mobilize high amounts of fat resulting in formation of acetyl coenzyme A, the citric acid cycle does not get blocked due to the high inflow of anaplerotic carbon in the form of oxaloacetate originating from the starch in the lactation diet. Hence, ketone formation was prevented (Laffel, 1999) and VFI was not decreased due to the nausea which can be caused by ketone bodies in the blood stream (Alsop et al., 1994). Using starch instead of fat as main energy source in the diet could have prevented this negative cascade. In agreement with this hypothesis, earlier research pointed out that sows on a lactation diet with fat as main energy source became more catabolic when compared to sows on a starch based lactation diet (Nelssen et al., 1985b). Another hypothesis is that in the present study the increased fat reserves at farrowing were combined with increased protein mass. Sinclair et al. (2001) reported that in primiparous sows with high back fat levels at farrowing, lactational feed intake was only suppressed when protein reserves were low. In the present trial, weight loss, which represents loss of fat, water, and protein (Guedes and Nogueira, 2001), was similar for all sows whereas back fat loss, representing mobilization of fat reserves only, augmented with increasing body

condition. Therefore, it can be assumed that fat sows mobilized proportionally less protein.

The differences in back fat loss between different body condition groups agrees with earlier research reporting elevated mobilization of back fat throughout lactation when sows had higher fat reserves at farrowing (Prunier et al., 2001; Revell et al., 1998b; Schenkel et al., 2010). However, in line with Neil (1996), feeding sows *ad libitum* during the peripartal period reduced the amount of back fat mobilized. During the peripartal period, sows with back fat thickness of less than 22 mm at late gestation even gained back fat when fed to appetite.

The metabolic profiles of the sows showed increased muscle catabolism, represented by elevated CREA levels (Hulten et al., 2002b; Yang et al., 2009), starting from day 112 of gestation. Despite the elevated feed intake of the *ad libitum* fed sows, mobilization of lean tissue could not be prevented or reduced, except on day 112 of gestation. At that day, the amount of feed offered to the restricted fed sows was only 21.7 % (1.6 kg versus 7.38 ± 2.04 kg) of the amount consumed by the sows fed *ad libitum*, and indeed the *ad libitum* fed sows mobilized less lean tissue. When severely restricting feed intake of sows until 3 days post-farrowing (Neil, 1996), halving lactational energy intake (Reese et al., 1984), or lowering lactational lysine intake (Yang et al., 2009), slightly higher CREA levels were reported. However, when considering moderate differences in lactational energy intake in primiparous sows, no differences in CREA levels could be recorded (Nelssen et al., 1985a). It can thus be assumed that although applying a restricted peripartal feeding scheme, feed intake was sufficient to prevent excessive mobilization of lean tissue. The lack of effect of body condition on this parameter indicates that all sows mobilized comparable amounts of lean tissue, indicating that mobilization of lean and fat tissue occurs independently from body condition (Hulten et al., 1993). This explains why no relation between the loss of body weight and the amount of back fat mobilized was detected. It is, however, possible that elevated CREA levels resulting from lean tissue catabolism were efficiently excreted in the urine. Therefore, it would have been usefull to determine CREA in the urine so renal clearance could be determined and a better estimate of muscle breakdown could be made.

Whereas CREA levels pre-prandially and 2 h post-prandially did not differ significantly (Mosnier et al., 2010), this was not the case for NEFA levels. In most studies NEFA levels were measured after an overnight fasting period of

several hours. In the present study, they were determined 2 h post-prandial implying a serious reduction in NEFA compared to pre-prandial levels (Mosnier et al., 2010; Theil et al., 2012). It, however, appears that at day 1 post-farrowing NEFA levels show an apex as a result of elevated mobilization of fat reserves (Mosnier et al., 2010) possibly as a result of lowered feed intake at the moment of farrowing (Le Cozler et al., 1999) combined with high energy demands for the onset of lactation. Again at day 112, when the difference in feed intake between the ADLIB and the STANDARD group was largest, the restricted fed sows were more catabolic than those fed to appetite. Despite what could be expected based on previous research (Revell et al., 1998b), fatness of sows did not influence NEFA levels. Probably, the absence of an effect of condition on NEFA levels is due to the post-prandial blood samplings. When considering TG, for which pre-prandial and 2 h post-prandial results are more comparable (Mosnier et al., 2010), a clear effect of body condition was present. Up to day 109 of gestation TG levels of fat sows were markedly higher than TG levels of sows with less than 22 mm of back fat. Higher catabolism of fat tissue for fat sows (Revell et al., 1998b) could result in elevated blood TG levels as the liver is not capable of coping with the excessive TG, resulting in lowered clearance of TG. The decrease of TG throughout the peripartal period was in accordance with previous research (Mosnier et al., 2010) and most likely the result of the start of colostrum synthesis shortly before farrowing (Devillers et al., 2006). With the start of lactation, TG decreases even more due to the efficient extraction by the mammary gland (Dourmad et al., 2000). Whereas TG levels of fat sows fed restricted decreased rapidly with the onset of lactation, this was not the case for fat sows fed *ad libitum*. Based upon the higher TG levels of the latter sows and the lower weaning weight of their piglets, it can be assumed that total milk output of these sows, on an energy basis, was lower. Unfortunately, apart from piglet growth, no further estimation of milk production could be measured in our study set-up and also no milk samples for proximate analyses were taken. Similar to the inverse relation between TG levels in early lactation and piglet growth reported by Mosnier et al. (2010), sows with back fat thickness above 22 mm mobilize back fat during the peripartal period which they try to compensate during the rest of lactation at the expense of piglet growth. In accordance with present results in multiparous sows, also in fat primiparous sows reduced litter

growth was reported as a result of lower milk output (Revell et al., 1998a; Whittemore, 1996).

The profiles of the bone markers measured in the present study nicely fit within previous studies reporting profiles of CTX and OC in gestating and lactating sows (Lauridsen et al., 2010; Liesegang et al., 2005). The decrease in CTX levels towards day 109 of gestation suggests that mineralization of the fetal skeleton is almost completed resulting in relatively low need for calcium and other bone minerals. As previously mentioned, colostrum synthesis already started before farrowing (Devillers et al., 2006) resulting in increased demands for calcium and other bone related minerals. The present study showed that from day 112 onwards bone degradation rises again. As sow milk gradually increases in calcium and phosphorus content throughout lactation (Csapo et al., 1996) and bone tissue is the main reserve for these minerals (Giesemann et al., 1998), the further increase of CTX levels during early lactation was not surprising. In order to prevent demineralization of the sows' skeleton, shortly after parturition not only bone degradation but also bone formation increased. Due to the onset of lactation and uterus contractions during parturition, there is a very high calcium demand at the start of lactation (Mahan and Vallet, 1997). These high calcium requirements correspond to the nadir in OC levels at day 1 of lactation. Afterwards, bone formation increased again as shown by increasing OC levels. Except for higher CTX levels on day 112 of gestation associated with restricted feeding, peripartal feed intake did not affect either bone degradation or bone formation. In contrast to expectations, the increased dietary calcium and phosphorus intake of *ad libitum* fed sows did not affect the bone markers. This has not been studied previously in sows, but research in dairy cattle showed that, although the peripartal decrease in calcium correlated nicely to increased CTX (Kronqvist et al., 2011) and decreased OC (Sato et al., 2011) levels, the peripartal profile of these markers seems to be independent of calcium and phosphorus intake (Ekelund et al., 2006; Kronqvist et al., 2011; Moreira et al., 2009). It has been shown that fat mass of premenopausal women is negatively associated with bone turnover markers (Thomas et al., 2001). Furthermore, a relation between body condition and bone metabolism could have been expected as associations were found between the sows body condition and plasma leptin concentrations (Estienne et al., 2000; Mosnier et al., 2010), and between leptin and bone metabolism (Thomas et al., 2001; Wolf, 2008). In contrast to the

reported associations between fat mass and bone metabolism, this relation was not confirmed for peripartal sows in the present study.

Next to metabolites representing bone, fat, and muscle metabolism, metabolic activity can be estimated by thyroid hormones (Mostyn et al., 2006). The T_4 levels reach a nadir at day 109 of gestation whereas for T_3 levels this nadir occurred at day 112 of gestation. Afterwards T_4 levels remained lower in contrast to T_3 levels which increased until day 3 of lactation. This can be explained by an increased peripheral conversion of T_4 to T_3 by outer ring type I deiodination (Samanc et al., 2010). Thyroid hormones are not only involved in lipid and carbohydrate metabolism (Samanc et al., 2010) but they are also important in bone turn-over metabolism (Bassett and Williams, 2008; Gogakos et al., 2010). Given the similar profile of CTX and T_3, a catabolic effect of T_3 on bone tissue, resulting in elevated CTX, could be assumed similar to what has already been described in adult mice (Gogakos *et al.*, 2010). Although, obesity and overeating in humans resulted in an activation of outer ring deiodination of T_4 to T_3 (Biondi, 2010), the present trial failed to demonstrate an effect of feeding strategy or condition of peripartal sows on thyroid hormones. Thyroid hormones are also involved in establishing metabolic priority for lactation (Capuco et al., 2008) and consequently, PHS was associated with impaired thyroid action (Wagner, 1972).

Despite the lack of effect of *ad libitum* feeding on most measured metabolites, beneficial effects on piglet performance were recorded in the present study. Although increased peripartal feed intake did not improve growth during early lactation, it had a positive influence on piglet weaning weight on the premise that sows were not too fat at the time of transfer to the farrowing unit and placed on *ad libitum* feeding. These findings are consistent with earlier research reporting that feeding level around farrowing is related to the amount of body reserves mobilized (de Lange et al., 1980) and does not affect piglet birth weight (Clowes et al., 2003). However, when body condition is not optimal, the number of stillborn piglets increased, which is in line with what was reported earlier for primiparous sows (Whittemore, 1996).

6.5 Conclusion

Throughout the peripartal period, metabolism changes dramatically regardless of body condition or applied feeding strategy. Not only mobilization of fat reserves, but also mobilization of muscle and bone tissue, starts from day 112 of gestation

and progresses throughout early lactation. On the premise that back fat of sows is beneath 22 mm at late gestation, *ad libitum* feeding during the peripartal period can maximize lactational feed intake, hence, limiting excessive loss of body reserves, and improving litter growth and weaning weight.

Acknowledgements

Chapter 7

Functional fibres in parturition feed

Parturition feed of sows with functional fibres: carry-over effect on lactational feed intake

A. Cools, D. Maes, J. Buyse, K. Rochus, B. Wuyts, G.P.J. Janssens

Feeding gestating sows a diet high in fibre has a beneficial effect on lactational feed intake. However, little is known about the effect of high fibre diets during the peripartal period on feed intake and performance throughout lactation. Therefore, the present study investigated the effect of high fibrous parturition diets, containing either sugar beet pulp (SBP), wheat bran (WB), or raw potato starch (RS) as main fibre source, on feed intake and metabolism during lactation. A total of 95 sows was divided over three treatment groups and fed from day 109 of gestation until day 1 of lactation a parturition diet based on SBP (n = 32), WB (n = 31), or RS (n = 32). At day 2 of lactation all sows were fed the same lactation diet until weaning. Individual daily feed intake was recorded from day 109 of gestation until weaning, constipation was scored daily, back fat thickness was measured on day 106 and 113 of gestation, and at day 1, 7, 13, and 20 of lactation, and reproduction results were noted. Fasted blood samples were taken on day 109 of gestation and on day 2 and 21 of lactation for analyses of triglycerides (TG), non-esterified fatty acids (NEFA), creatinine (CREA), leptin, ghrelin, free amino acids and acylcarnitine profile. Total feed intake from day 109 of gestation until weaning was significantly higher for sows fed the SBP diet compared to the WB diet (P = 0.017). Differences in feed intake between fibre sources tended to be most apparent after farrowing when the same lactation diet was offered to all groups (P = 0.061). Both TG (P < 0.001) and NEFA (P = 0.023) showed a significant interaction time × diet, whereas CREA levels did not differ between time points or offered parturition diets. Leptin and ghrelin levels showed a significant drop after farrowing (P < 0.001) and a tendency towards lower leptin levels at weaning for the SBP compared to the WB diet (P = 0.083). Back fat thickness was significantly higher for sows in the RS group compared to the two others (P < 0.001). Although metabolic parameters and back fat thickness were affected by type of parturition feed, no differences in reproduction results between the groups could be detected. The results of the present trial showed that high fibre diets based on SBP, even when fed during a short period of time, positively influence lactational feed intake, probably regulated by decreased leptin concentrations. Although RS is not frequently used in practice so far, it can be a promising fibre source in diets for peripartal sows.

7 Functional fibres in parturition feed

7.1 Introduction

Including fibre in sow gestation diets has a positive influence on gut health (Renteria-Flores et al., 2008a; Varel and Pond, 1985) and performance of both lactating sows and piglets (Danielsen and Vestergaard, 2001; Guillemet et al., 2007; Matte et al., 1994; Quesnel et al., 2009; van der Peet-Schwering et al., 2003a; Vestergaard and Danielsen, 1998). Former research, applying a wide variety of raw materials as fibre sources, demonstrated that fibre type is of great importance in gestation diets: studies comparing soluble against insoluble fibres in gestation feed reported decreased energy digestibility with increasing levels of soluble fibre (Renteria-Flores et al., 2008a) without effects on litter size (Renteria-Flores et al., 2008b) but with lower piglet birth weight (Danielsen and Vestergaard, 2001). Sows lost less body weight throughout lactation in case of combined increase of soluble and insoluble fibres in the gestation diet (Renteria-Flores et al., 2008b). Besides classifying fibres as either soluble or insoluble, fibre fermentability may affect performance. Depending on the fibre type (Graham et al., 1986), adult sows are able to ferment rather large amounts of fibre in their hindgut (Le Goff and Noblet, 2001; Varel and Yen, 1997). This fermentation leads to VFA that can be absorbed in the blood stream (de Leeuw et al., 2004; Noblet and Le Goff, 2001; Serena et al., 2007) and influence the postprandial insuline and glucose response (de Leeuw et al., 2004; Quesnel et al., 2009; Ramonet et al., 2000). Non starch polysaccharides (NSP) based diets can be used by gestating sows as efficiently as starch rich diets (Rijnen et al., 2001; van der Peet-Schwering et al., 2002). Apart from what is chemically analyzed as fibre (sum of NSP and lignin (de Leeuw et al., 2008)), resistant starch is also not digested by mammalian enzymes (de Leeuw et al., 2008), not absorbed in the small intestine, and can be fermented in the hindgut (Martin et al., 1998). Therefore, this component is defined as a functional fibre (Millet et al., 2010). Despite all the beneficial effects of fibre in gestation diets, barely any research on the effect of fibre in peripartal diets has been conducted. Studies with low fibre diets around farrowing report an increase of the incidence of constipation at farrowing (Oliviero et al., 2009; Tabeling et al., 2003). Therefore, the aim of this study was to determine the effect of three different fibre sources frequently used in sow feed (sugar beet pulp, wheat bran, and raw

potato starch) on lactational feed intake, occurence of constipation, and the metabolism of sow. As already proven that high fibreous diets are beneficial for sows, no negative control (low in fibre) was included in the trial.

7.2 Materials and methods

The experimental design was approved by the Ethical Committee of the Faculty of Veterinary Medicine, Ghent University Belgium (EC2011/137) and by the Federal Public Service Health, Food Chain Safety and Environment, Belgium.

7.2.1 Animals, feeding, and experimental design

The experiment was performed at a commercial farrow-to-finish herd in West Flanders, Belgium. The herd accommodated 500 sows (Landrace) in a 4-week batch production system. All sows were housed according to Belgian and EU legislation for sow housing. All stables were equipped with automated climate control systems and mechanical ventilation, light was provided during 12 h per day. From insemination (artificial insemination with Belgian Pietrain semen) until day 28 of gestation, sows were individually housed in crates, afterwards they were moved to group housing until day 108 of gestation. During the period from insemination until day 108 of gestation, all sows received the same commercial gestation feed (890.3 g/kg dry matter, 52.0 g/kg crude ash, 145.8 g/kg crude protein, 40.7 g/kg crude fat, 64.3 g/kg crude fibre, 8.80 MJ/kg NE, all on as-fed basis), provided as a meal and following a restricted feeding scheme. At day 108 of gestation sows were removed from the group housing, washed with warm water and then housed in individual farrowing crates until weaning (day 21 of lactation). From one group of the batch production system all sows with parity 1 to 7 were selected (95 sows). The 95 selected sows were divided randomly into three feed treatment groups. The first group (n = 32, parity 3.1 ± 1.9, back fat 18.0 ± 2.9 mm) received a parturition feed with sugar beet pulp (SBP) as main fibrous ingredient, the second group (n = 31, parity 3.0 ± 1.9, back fat 18.7 ± 2.8 mm) was fed a parturition feed with elevated levels of wheat bran (WB) and the third group (n = 32, parity 3.7 ± 1.8, back fat 17.7 ± 2.8 mm) received a parturition feed with raw potato starch as a source of resistant starch (RS) as main ingredient (Table 7.1). All parturition diets were formulated isocaloric and with similar levels of crude fibre but different levels of NDF, ADF, and ADL, and were fed from day 109 of gestation until one day after farrowing. At day 2 of

lactation, all sows shifted from the parturition diet to the same commercial lactation feed (894.2 g/kg dry matter, 60.7 g/kg crude ash, 162.7 g/kg crude protein, 41.7 g/kg crude fat, 63.1 g/kg crude fibre, 9.47 MJ/kg NE, all on as-fed basis) for all groups until weaning. During the peripartal period sows were fed twice daily and when shifted to the lactation diet, all sows were fed three times a day. Portions fed to every sow were adjusted to appetite at every feeding. The amount of feed offered at every feeding as well as the left overs were recorded and based on these results, the daily feed intake of each sow was calculated.

Table 7.1 Composition of the three experimental diets, one based on sugar beet pulp (SBP), a second based on wheat bran (WB), and a third with raw potato starch as fibre source (RS).

	SBP	WB	RS
Ingredients			
Wheat	20.80	20.59	20.97
Barley	20.70	20.49	20.87
Soy bean meal	11.33	11.21	11.42
Sugar beet pulp	21.25	6.56	6.68
Wheat bran	4.23	13.82	4.26
Maize starch	0.00	4.80	0.00
Resistant potato starch	0.00	0.00	14.75
Soy hulls	8.17	8.09	8.24
Sugar beet molasses	3.31	3.28	3.34
Flaxseed	1.66	1.64	1.67
Pork lard	1.73	1.71	1.74
Limestone	1.39	1.38	1.40
Fish oil	0.44	0.44	0.44
Dextrose	2.57	3.28	0.00
Magnesium sulfate	0.00	0.29	1.80
Synthetic amino acid premix[1]	0.37	0.37	0.37
Mineral premix[2]	1.56	1.56	1.56
Vitamin premix[3]	0.35	0.35	0.35
Choline chloride	0.14	0.14	0.14
Nutrient content[4]			
Dry matter	87.8	88.3	87.3
Crude ash	7.48	8.15	8.45
Crude protein	13.8	14.8	13.4
Crude fat	3.98	3.93	3.45
Crude fibre	12.4	11.2	12.4
NDF	33.8	20.0	16.9
ADF	10.9	8.88	7.39
ADL	1.46	1.40	1.01
NE (MJ/kg)[5]	9.50	9.50	9.50

[1] Premix containing 67.6% L-lysine, 18.9% L-threonine, and 13.5% DL-methionine.
[2] Premix containing 28.000% vit C, 0.403% vit A, 0.002% vit D_3, 10.14% Vit E, 0.073% vit K_3, 0.073% vit B_1, 0.365 % vit B_2, 0.547% vit B_3, 1.094% vit PP, 0.219% vit B_6, 0.004% vit B_{12}, 0.015% biotin, and 0.109% folic acid.
[3] Premix containing 42.30% monocalcium phosphate, 26.30% sodium chloride, 23.70% magnesium phosphate, 1.13% calcium, 1.26% sulpher, 1.25% iron, 0.42% manganese, 0.84% zinc, 0.13% copper, 0.02% iodine, 1.25% iron sulphate, 0.02% calcium iojate, 0.13% copper sulphate, 0.41% manganese oxide, 0.84% zinc sulphate.
[4] Nutrient content reported on as-fed basis.
[5] Calculated using feed formulation software and based on feedstuff tables of CVB 2007 (Productschap Diervoeder, The Hague, The Netherlands).

Throughout the entire trial, all sows had *ad libitum* access to fresh drinking water (drinking nipple with flow 2 l/min). During the period of feeding the parturition diet (from day 109 of gestation until day 1 of lactation), the presence of feces behind each sow was recorded every morning by the same person and subsequently, the feces were removed. Based on Oliviero et al. (2009), the grade of constipation during the peripartal period was assessed as no constipation (no days without feces production), mild constipation (one or two consecutive days without feces production) and severe constipation (more than three consecutive days without feces production).

7.2.2 Blood sampling

All blood samplings were performed at 7.00 in the morning, after an overnight fasting period of 12 h. At day 109 of gestation, at day 2 of lactation and at weaning a 20 ml blood sample was taken by puncturing the *vena jugularis*. Immediately after sampling, each blood sample was divided into 3 subsamples (9 ml on K_3EDTA, 6 ml on lithium heparin and 5 ml on serum clot activator) and stored on ice water (4 °C) for 3 h until serum or plasma was separated by centrifugation (10 min, 4 °C, 1000 x *g*). Both serum and plasma samples were stored frozen (-20 °C) until further analyses. Blood sampling was succesfull at each time point for all sows of the SBP and WB group and for 29 of the 32 sows of the RS group.

7.2.3 Chemical analyses

Feed samples were analyzed in accordance with methods determined by the Association of Offical Analytical Chemists. Creatinine (CREA) and triglycerides (TG) in plasma samples were determined spectrophotometrically (Ultrospec IIE, LKB Biochrom Ltd., Cambridge, United Kingdom) by adding picric acid and sodium hydroxide (Randox Crea, Randox Laboratories Ltd., Crumlin, United Kingdom) for creatinine analysis and by adding lipase, glycerol-kinase, glycerol-3-phosphate oxidase and peroxidase (Randox Trigs, Randox Laboratories Ltd., Crumlin, United Kingdom) for TG analysis. Furthermore, plasma samples were analyzed spectrophotometrically (EZ Read 400 Microplate Reader, Biochrom Ltd., Cambridge, United Kingdom) to determine non-esterified fatty acids (NEFA) by adding acyl-CoA synthetase, acyl-CoA oxidase and peroxidase (Randox NEFA, Randox Laboratories Ltd., Crumlin, United Kingdom). Leptin was measured with a multispecies RIA kit (Multi-Species Leptin RIA Kit, Merck Millipore, Huissen, The

Netherlands) using a guinea pig anti-multi-species leptin antibody and ^{125}I-human leptin label. This multi-species kit had a specificity of 67 % for porcine leptin. For ghrelin analysis, a porcine RIA kit (Porcine Ghrelin RIA KIT, Phoenix Pharmaceuticals Inc., Karlsruhe, Germany) was used. This commercial kit used rabbit antibody specific for the porcine ghrelin peptide and ^{125}I-porcine ghrelin label. All samples were run in the same assay to avoid interassay variations. Acylcarnitine profile (acetyl- (C2), propionyl- (C3), butyryl- + isobutyryl- (C4), isovaleryl- + 2-methylbutyryl- (C5), tiglyl- + 3-methylcrotonyl- (C5:1), 3-hydroxybutyryl- (3OHC4), 3-hydroxyisovaleryl- + 2-methyl-3-hydroxybutyryl- (3OHC5), malonyl- (C3DC), methylmalonyl- (C4DC), glutaryl- (C5DC), and 3-hydroxy-3-methylglutarylcarnitine (C6DC)) of serum samples was determined using quantitative electrospray tandem mass spectrometry (Zytkovicz et al., 2001). With the same method also specific free amino acids (valine, leucine, methionine, phenylalanine, tyrosine, glycine, L-alanine, ornithine, and citrulline) in the serum were measured.

7.2.4 Sow back fat thickness and litter weight

Back fat thickness of each sow was measured on the P2 position (Maes et al., 2004) using a Renco Lean-Meater (S.E.C. Repro Inc., Ange-Gardien-de-Rouville, Québec, Canada). For each sow, back fat levels were measured left and right from the spinal column and an average was calculated per sow. Measurements were performed on day 106 and day 113 of gestation, and on days 1, 7, 13, and 20 of lactation. Furthermore, each litter was weighed on days 1, 7, 13, and 20 of lactation and the average piglet weight was calculated by dividing the total litter weight by the number of piglets present at the moment of weighing. After day 1 of lactation piglets were cross-fostered within treatment groups if the number of piglets was higher than the number of functional teats at the udder of the sow.

7.2.5 Reproductive performance

For each sow, gestation length, numbers of live born and stillborn piglets were recorded. The percentage of live born and stillborn piglets was calculated based on the number of total born piglets. To investigate the influence of the peripartal feeding on the ovulation rate after weaning, total number of piglets born (live born, still born, and mummies) in the next reproduction cycle was recorded for 75 of the 95 sows (SBP 26 sows, WB 24 sows, and RS 25 sows).

7.2.6 Statistical analyses

All data were entered in Microsoft® Office Excel® 2007 (Microsoft Corporation, Redmond, Washington, USA) prior to statistical analyses, which were performed using SPSS 20.0 (SPSS, Chicago, Illinois, USA). Homogeneity of variance and normality of all data was tested using a modified Levene's test and a Kolmogorov-Smirnoff test, respectively. For all statistical analyses, significance was set at $P < 0.05$. Feed intake results of sows were all adjusted to actual farrowing date. These results were analyzed using repeated measures analyses of variance (ANOVA) with time as within-subject and treatment as between-subject factor. Differences between treatment groups were detected using a post-hoc Tukey test. Constipation scoring was summarized in a singly ordered contingency table with constipation scoring as the ordered columns and treatment as the unordered rows. Differences between treatment groups were detected using an exact Kruskal-Wallis test. The blood parameters were analyzed using repeated measures ANOVA with time as withing-subject and treatment as between-subject factor. Evolution of back fat thickness was subjected to repeated measures ANOVA with time as within-subject factor, treatment as between-subject factor and the first back fat measurement (d 106 of gestation) as covariate. Significant differences between the within-subject factors were tested using least significant differences (LSD) test with Bonferroni correction. Changes of back fat between day 106 of gestation and day 1 of lactation, and between day 1 of lactation and weaning were calculated for each sow and together with reproductive performance data (gestation length, total number born, liveborn, stillborn, % liveborn, % stillborn, and total number born in the next cycle) subjected to one way ANOVA with feed treatment as factor. All data were reported as mean ± standard deviation (SD).

7.3 Results

The overall feed intake of the sows during the period in the farrowing room (from day 109 of gestation until weaning) was significantly influenced by the type of parturition diet ($P = 0.017$, Figure 7.1). However, it was not the feed intake of the parturtion diet that was affected ($P = 0.443$) but that of the lactation diet afterwards ($P = 0.061$). Sows that where offered the SBP diet consumed more feed throughout the lactation period than sows offered the WB diet during the

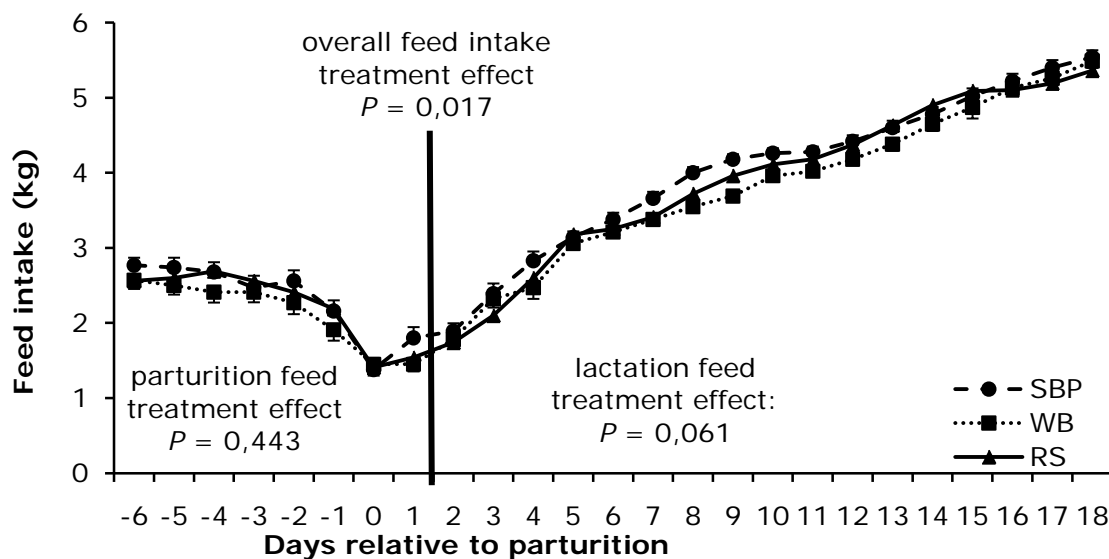peripartal period. Also no hypophagia could be observed in any of the treatment gropus.

Figure 7.1 Feed intake of sows (mean ± SD) fed a sugar beet pulp based parturition diet (SBP), a wheat bran based parturition diet (WB), or a parturition diet with raw potato starch (RS) as fibre source. Total feed intake during the entire period in the farrowing room (gestation day 109 until weaning) was significantly higher for sows fed the SBP diet compared to those fed the WB diet (P = 0.017).

Constipation scoring was not significantly different between the three diets, although in the RS group numerically more sows had no constipation and less sows had mild constipation around farrowing when compared to the two other diets (Figure 7.2).
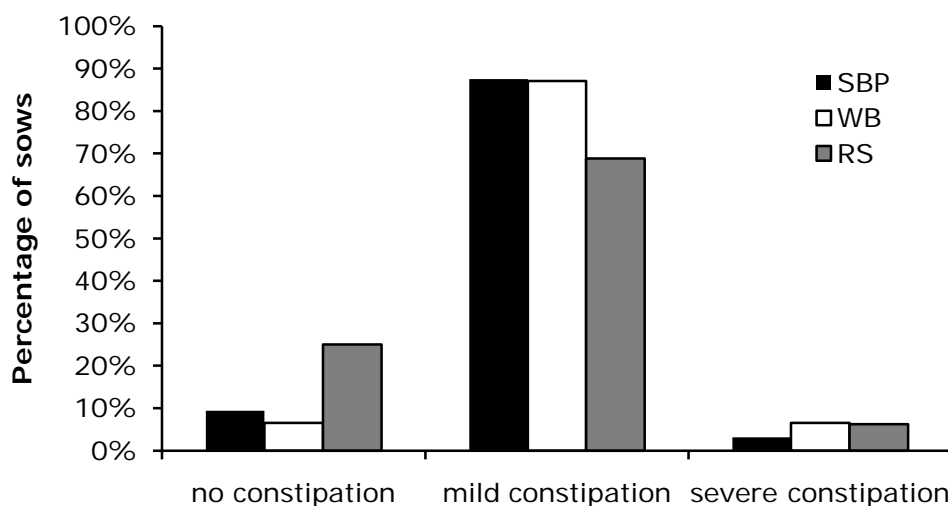
Figure 7.2 Incidence of constipation around farrowing (5 days before until 2 days after parturition) in sows fed a parturition diet based on sugar beet pulp (SBP), wheat bran (WB), or raw potato starch (RS) (P = 0.196). The scoring of constipations was no constipation (no days without feces output), mild constipation (sows did not produced any feces for one or two consecutive days), and severe constipation (more than two consecutive days no feces produced).

Both TG and NEFA concentrations in the blood showed a significant interaction between time of measurement and diet ($P < 0.001$ for TG and $P = 0.023$ for NEFA, Figure 7.3,).
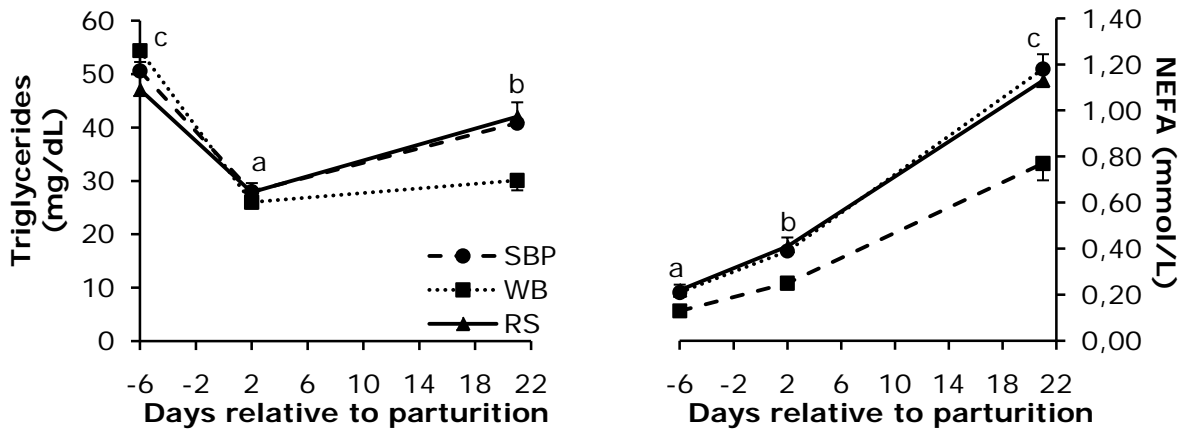

Figure 7.3 TG profile (left) and NEFA profile (right) of sows fed different fibre sources during the peripartal period (mean ± SEM). For TG there was a significant interaction between time and treatment with a difference at weaning (3rd time point) between the wheat bran based parturtion diet (WB), and the sugar beet pulp based (SBP) and raw potato starch based (RS) diet ($P < 0.001$). For NEFA there was a significant interaction between time and treatment with a difference at the beginning of lactation (2nd time point) and at weaning (3rd time point) between WB, and SBP and RS ($P = 0.023$).Time points (days relative to parturition) with a different letter differ significantly from each other regardless treatment ($P < 0.001$).
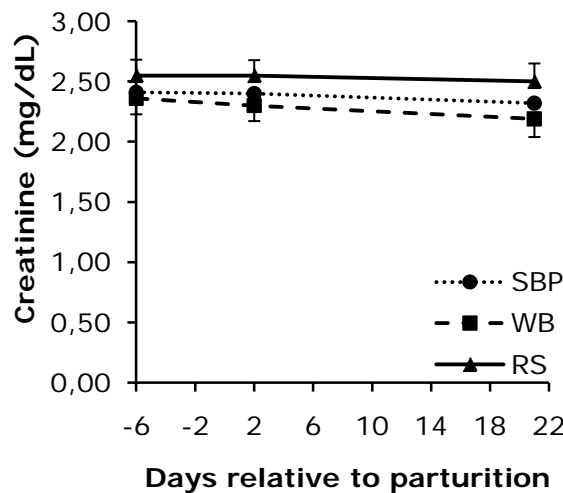
Figure 7.4 CREA profile of sows (mean ± SEM) fed a parturition diet based on sugar beet pulp (SBP), wheat bran (WB), or raw potato starch (RS). No significant effects of either time or diet could be detected ($P > 0.05$).

The CREA levels were not significantly influenced by time point or diet (Figure 7.4). Leptin and ghrelin profiles were influenced by time, showing a clear drop after parturition (Figure 7.5). For leptin there tended to be an interaction between time × diet ($P = 0.083$). The sows of the SBP group tended to have leptin levels lower at weaning compared to the sows of the WB group. Regardless

of dietary treatment, ghrelin levels of all sows were very low just after farrowing and increased towards weaning.
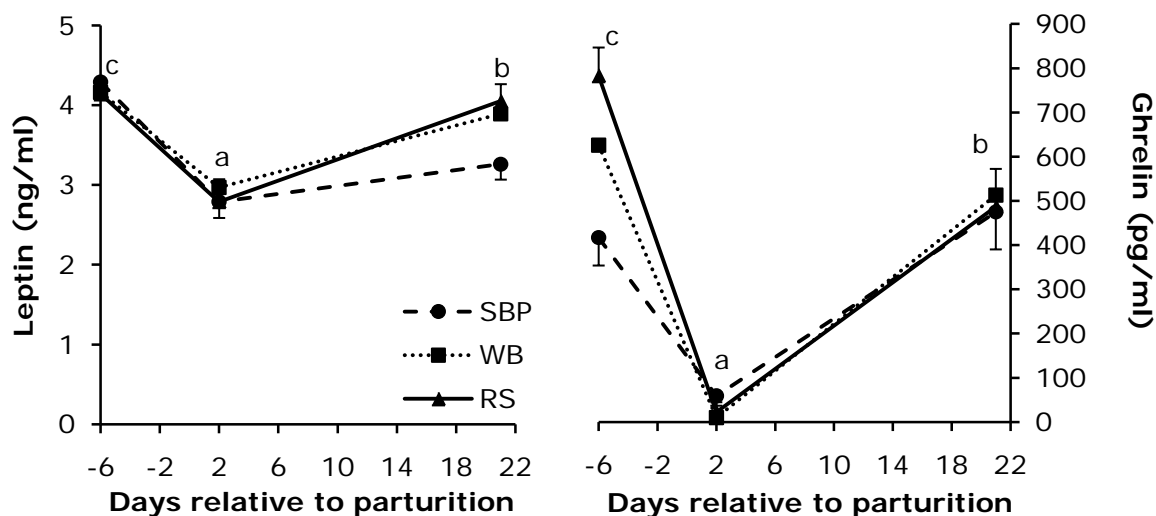

Figure 7.5 Leptin (left) and ghrelin (right) levels for sows fed three different parturition diets (based on sugar beet pulp (SBP), wheat bran (WB), or raw potato starch (RS)). Both hormones (mean ± SEM) showed a time dependent profile with different letters at different time points indicating significant differences ($P < 0.001$). Leptin (left graph) tended to be affected by the interaction between time × diet ($P = 0.083$) with lower leptin levels at weaning for SBP compared to WB.

Except for C3 and C5DC, all acylcarnitines measured showed a time dependent profile (Table 7.2). Similarly, profiles of all free amino acids measured in sows' serum changed over time. Furthermore, there was a time × diet interaction for free leucine ($P = 0.002$) and glycine ($P = 0.009$) as well as for C4 ($P = 0.001$) and 3OHC4 ($P = 0.009$). Both C4 and 3OHC4 increased after sows were fed the RS diet. Sows that were fed the WB diet had higher free methionine concentrations compared to the two other diets ($P = 0.002$) and higher free L-alanine levels than the RS fed sows ($P = 0.021$). For sows fed the RS diet C6DC concentrations were higher than for sows fed the SBP parturition diet ($P = 0.024$).

Table 7.2 Preprandial concentrations of free amino acids and acylcarnitine profile measured in sows before (gestation day 109) and after (lactation day 2) they were fed a parturition diet (parturition diet rich in sugar beet pulp (SBP), wheat bran (WB), or raw potato starch (RS)), and at weaning.

	Gestation day 109				Lactation day 2				Weaning				P-value[1]		
	SBP	WB	RS	SEM	SBP	WB	RS	SEM	SBP	WB	RS	SEM	T	T × F	F
Free amino acids[1]															
Valine	61.1	59.6	58.8	1.0	56.9	61.6	62.4	1.5	53.0	53.6	51.5	1.0	<0.001	0.199	0.519
Leucine	355.4	359.9	349.9	5.4	363.2	375.3	381.7	6.9	384.6	371.5	331.6	6.1	0.039	0.002	0.345
Methionine	42.9	48.0	45.3	1.2	59.9	58.4	58.1	1.5	37.2	45.9	36.2	1.2	<0.001	0.165	0.022
Phenylalanine	69.5	73.5	76.0	1.7	83.8	89.1	89.0	2.3	50.8	52.7	47.2	1.0	<0.001	0.394	0.382
Tyrosine	65.5	69.8	69.0	1.8	93.5	96.8	90.0	2.9	51.0	55.2	53.9	1.7	<0.001	0.796	0.513
Glycine	719.2	708.3	724.9	12.8	629.6	727.5	618.0	15.6	544.6	621.3	523.2	13.6	<0.001	0.009	0.020
L-alanine	784.7	818.6	759.7	14.8	664.6	709.4	661.0	20.7	466.0	584.7	477.0	15.7	<0.001	0.515	0.021
Ornithine	67.7	69.8	77.0	2.7	79.8	97.5	83.1	3.8	47.6	46.8	50.0	2.2	<0.001	0.172	0.325
Citrulline	102.6	91.7	111.3	3.4	99.7	112.8	110.2	4.7	103.8	115.7	131.3	5.0	0.012	0.112	0.148
Acyl-carnitines[2]															
C2	2.8	2.8	2.8	0.05	3.8	3.8	4.4	0.1	2.6	2.7	2.8	0.04	<0.001	0.132	0.108
C3	0.12	0.09	0.08	0.008	0.09	0.08	0.09	0.009	0.10	0.12	0.08	0.009	0.451	0.256	0.390
C4	0.36	0.24	0.21	0.02	0.16	0.18	0.24	0.01	0.43	0.35	0.36	0.02	<0.001	0.001	0.005
C5	0.075	0.090	0.096	0.005	0.090	0.090	0.150	0.011	0.056	0.065	0.064	0.004	<0.001	0.109	0.068
C5:1	0.055	0.039	0.043	0.003	0.027	0.035	0.050	0.004	0.057	0.044	0.063	0.005	0.009	0.122	0.060
3OHC4	0.032	0.020	0.026	0.002	0.030	0.030	0.040	0.003	0.027	0.034	0.023	0.002	0.012	0.009	0.779
3OHC5	0.059	0.054	0.061	0.003	0.047	0.057	0.056	0.003	0.060	0.059	0.073	0.003	0.042	0.432	0.179
C3DC	0.013	0.012	0.012	0.001	0.015	0.011	0.021	0.001	0.010	0.010	0.010	0.001	0.015	0.068	0.138
C4DC	0.036	0.035	0.035	0.002	0.033	0.027	0.038	0.002	0.043	0.040	0.047	0.002	<0.001	0.500	0.192
C5DC	0.019	0.017	0.017	0.001	0.010	0.020	0.020	0.001	0.015	0.015	0.022	0.001	0.470	0.055	0.392
C6DC	0.022	0.037	0.045	0.005	0.040	0.030	0.050	0.004	0.020	0.030	0.030	0.002	0.007	0.280	0.024

[1] P-values for time effect (T), feed treatment (F), and the interaction time × feed treatment (T × F).
[2] Expressed as µmol/l.
[3] Acylcarnitine profile expressed as µmol/l (acetyl- (C2), propionyl- (C3), butyryl- + isobutyryl- (C4), isovaleryl- + 2-methylbutyryl- (C5), tiglyl- + 3-methylcrotonyl- (C5:1), 3-hydroxybutyryl- (3OHC4), 3-hydroxyisovaleryl- + 2-methyl-3-hydroxybutyryl- (3OHC5), malonyl- (C3DC), methylmalonyl- (C4DC), glutaryl- (C5DC), and 3-hydroxy-3-methylglutarylcarnitine (C6DC)).
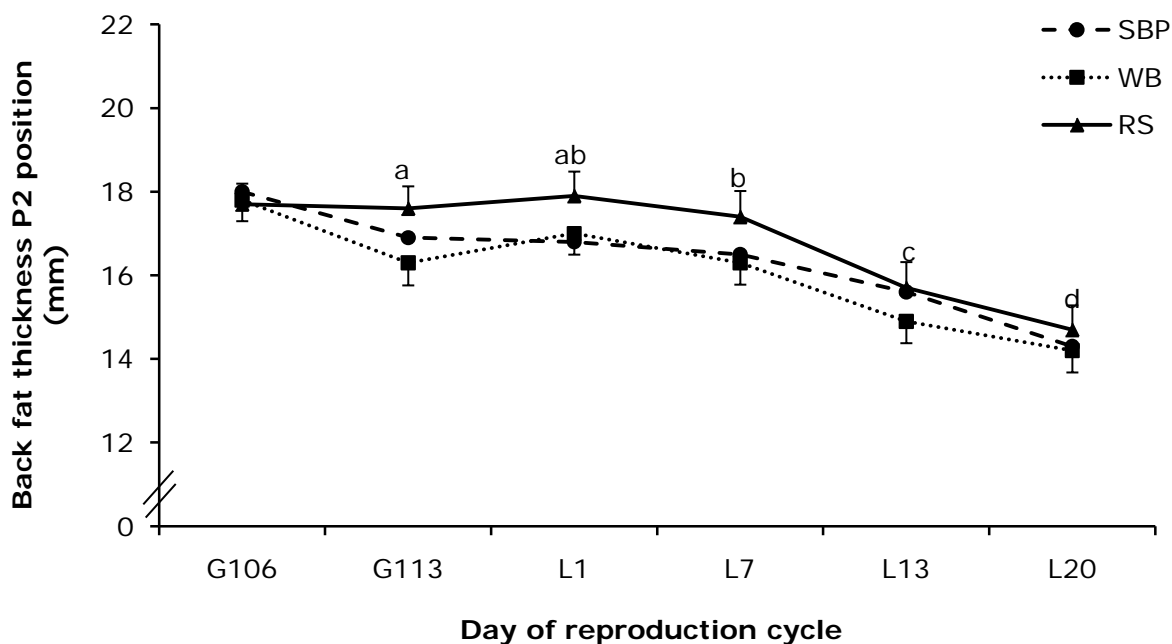
Figure 7.6 Evolution of back fat at the P2 position (mean ± SD) for sows fed a parturition diet with sugar beet pulp (SBP), wheat bran (WB), or raw potato starch (RS) as main fibrous ingredient. Time points with a different letter differ significantly from each other (P = 0.002). Sows fed the RS diet had significant higher back fat than those fed the SBP or WB diet (P = 0.001).

Back fat thickness throughout the peripartal and the lactation period is presented in Figure 7.6. Besides a significant change of back fat over time (P = 0.002), there was a significant difference between the back fat evolution of the RS group versus the SBP and WB groups (P = 0.001). More specifically, during the peripartal period (gestation day 106 until lactation day 1) back fat decreased with 1.16 ± 1.24 mm for the SBP group which was significantly lower compared with the RS group for which back fat increased with 0.19 ± 1.73 mm during the same period (P = 0.005). The WB group had a back fat decrease of 0.77 ± 1.89 mm during this period which was not significantly different from the two other groups. During lactation (lactation day 1 until weaning), back fat of all groups decreased, but the decrease was not affected by the dietary treatment during the peripartal period (SBP -2.55 ± 1.74 mm, WB -2.84 ± 1.84 mm, RS -3.20 ± 1.89 mm). As could be expected, piglet weight and litter weight significantly increased over time (P < 0.001, Figure 7.7) but without any effect of treatment. Also reproductive performance of sows and number of piglets born in the next cycle were not affected by the treatment (Table 7.3).
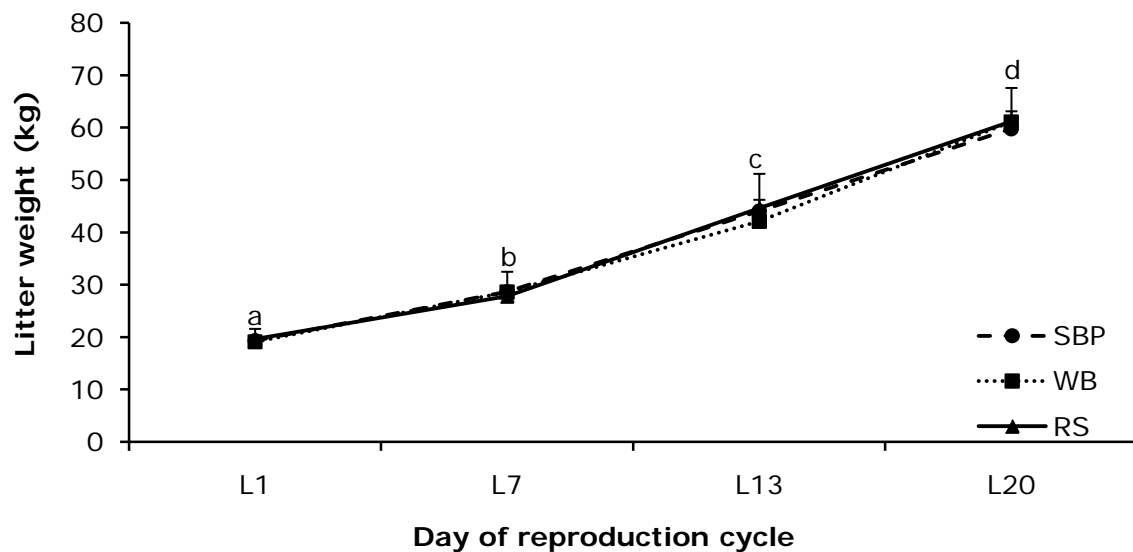
Figure 7.7 Evolution of litter weight of sows fed with three different types of parturition diet (high in sugar beet pulp (SBP), high in wheat bran (WB), or high in raw potato starch (RS). Litter weight increased significantly over time ($P < 0.001$, different letters indicate significantly different time points) without any effect of the diet.

Table 7.3 Reproduction results of sows fed different fibre sources (sugar beet pulp SBP, wheat bran WB, or raw potato starch RS) during the peripartal period

	SBP	WB	RS	SEM	*P*
Gestation length	115	115	115	0.1	0.824
Total born	13.6	13.6	14.0	0.2	0.718
Liveborn	13.0	13.0	13.2	0.3	0.966
% liveborn[1]	96.1	95.3	94.2	0.9	0.668
Stillborn	0.6	0.6	0.8	0.1	0.558
% stillborn[2]	3.9	4.7	5.8	0.9	0.668
Weaned piglets	11.4	11.2	11.5	0.1	0.272
Total born subsequent cycle	14.2	14.9	13.5	0.4	0.370

[1] Calculated as number of live born over total born.
[2] Calculated as number of stillborn over total born.

7.4 Discussion

The present trial showed a carry-over effect of peripartal feed intake on lactational feed intake. Former research has pointed out that there is a clear inverse relationship between feed intake during gestation and lactation (Dourmad, 1991). Other reports stated that it was not the actual gestational feed intake, but the body condition at farrowing that strongly correlates with total feed intake throughout lactation (Prunier et al., 2001; Revell et al., 1998b). The higher the body fatness of sows at farrowing was, the lower their voluntary feed intake during lactation. However, in the present trial, feed intake throughout gestation was equal for all sows and also body condition at the beginning of the peripartal period was equally distributed over the treatment groups. Therefore,

the effect of the composition of the parturition diet on lactational feed intake is at play. The positive carry-over effect of feeding SBP based parturtion diet on lactational feed intake is consistent with the results of Quesnel et al. (2009) and of Guillemet et al. (2006), although, in the present trial sows were fed the SBP diet for a much shorter time period (9 days). In these studies a bulky gestation diet, which resembles in terms of composition mostly the SBP diet in the present trial, was associated with an increased feed intake throughout lactation when compared to a low fibre gestation diet. Whereas SBP is a bulky fibre with a high water binding capacity, WB has a high bulkiness but with only a moderate water binding capacity, and RS has both low bulkiness and water binding capacity (de Leeuw et al., 2008). One of the hypotheses why SBP improves VFI is that ingestion of this fibre with high swelling properties results in distention of the stomach. This may lead to immediate postprandial satiety (de Leeuw et al., 2008), and afterwards, to higher VFI. Another hypothesis is that the pectin, which is abundant in sugar beet pulp (McDonald et al., 2011), increase the sow's feeding motivation. This phenomenon has been described preciously in fully grown, not reproducing sows (Souza da Silva et al., 2012). The mechanism how pectin may increase feeding motivation in a more or less dose responsive manner is not clear yet. The lowered leptin levels at weaning in the present trial support the hypothesis that the SBP parturition diet influences lactational feed intake, at least partially, via central humoral pathways. How the SBP diet was able to alter leptin levels remains unclear. The results of the present study also confirm previous research indicating that leptin and ghrelin function independently from each other (Sirotkin and Meszarosova, 2010). The decreased ghrelin levels shortly after parturition correspond with the decreased VFI around farrowing but unfortunately, this does not clarify how lactational feed intake was affected by peripartal feeding. The question remains whether the decreased ghrelin levels around farrowing result in a lower VFI or that the lowered ghrelin levels were a result of a reduced energy intake of the sows during this period. Given that earlier research in growing pigs indicated that ghrelin levels were correlated more closely with energy balance than with actual VFI (Scrimgeour et al., 2008), support this second hypothesis. Another alternative hypothesis is that ghrelin secretion was inhibited by short-chain fatty acids (SCFA) circulating in the blood as a result of hindgut fermentation as was the case in wethers (Fukumori et al., 2011). The three fibre sources used in the present trial not only differed in

bulkiness and water holding capacity, but they also had a different fermentability. Both SBP and RS are highly fermentable (de Leeuw et al., 2008), resulting in the production of SCFA. Research showed that hindgut fermentation of SBP mainly results in acetate and propionate (Anguita et al., 2007), whereas RS fermentation produces mostly butyrate (Martin et al., 1998; van der Meulen et al., 1997). Research in humans pointed out that WB is only partly fermented and only after a long adaptation period of 12 months (Freeland et al., 2010) resulting mainly in acetate production. Increased production of butyrate by the hindgut is, however, difficult to measure (Millet et al., 2010). The butyrate generated from the fermentation of RS will be used by the colonocytes as main energy source (van der Meulen et al., 1997) and converted over 3-hydroxy-butyryl-CoA into acetyl-CoA through the process of β-oxidation (Roediger, 2004). The remaining 3-hydroxy-butyryl-CoA that was not used by colonocytes enters the blood stream as ketone bodies (Livesey and Elia, 2004) which will then promote lipogenesis (Rémésy et al., 2004). The profile of the different acyl-carnitines reflects how the different diets affected the acyl-CoAs (Brass and Hoppel, 1980; Ramsay, 1999). For all diets, the C2 levels increased after feeding the different parturition diets, suggesting that all three diets fermented in the hindgut which resulted in an increase in acetate production. Levels of both C4 and 3OHC4 increased after feeding RS diets, possibly as a result of butyrate production. This is an indication that these acylcarnitines were suitable to detect differences in butyrate production.

Elevated CREA levels in producing sows are a clear indicator of muscle catabolism (Yang et al., 2009). It can, therefore, be concluded that none of the sows in the present trial were catabolizing lean tissue. As metabolites involved in fat metabolism were measured at a fasted state, they represent mobilization of body fat. A summary of different studies in rats reported that TG levels decreased after consumption of WB whereas pectin did not affect serum TG levels (Anderson and Hanna, 1999). In the present trial, however, all fibrous diets succeeded to decrease fasted TG levels by 50% after a period of 10 days. As already discussed, all diets fermented in the hindgut resulted in the production of SCFA which can be used as alternative energy source (Jorgensen et al., 2007). At weaning, both TG and NEFA of the WB fed sows were lower compared to the SBP and RS fed sows which suggest that the energy balance of the WB group was less negative than the other two sources. As loss of back fat

was not different between the different groups, another explanation for this difference is warranted. Possibly, the metabolism of sows fed high fermentable fibres throughout the peripartal period (SBP and RS group) was altered towards an energy metabolism more relying on the use of SCFA. When sows were shifted to the lactation diet, SCFA production decreased resulting in a more negative energy balance for the SBP and RS fed sows. Also the use of glycine as an alternative substrate for the citric acid cycle via conversion of glycine into pyruvate over serine (Michal, 1999) could explain the altered fat metabolism at weaning. The higher fasted glycine levels for WB fed sows, both in the beginning of lactation and at weaning, suggest that glycine in these sows is used as an alternative energy source for glucose. The elevated L-alanine for the WB fed sows confirms this hypothesis as this amino acid is a side product of the conversion of glycine into pyruvate (Michal, 1999). Increased concentrations of free leucine accompagnied by elevated concentrations of its metabolites C5, C5:1 and C6DC after feeding sows the RS diet points towards the use of leucine as an energy source in the citric acid cycle. However, further research should clarify how the amino acid metabolism was affected by hindgut fermentation in sows.

Peripartal diets were formulated isocalorically and peripartal feed intake was equal for all treatment groups. However, the back fat gain in the RS group can be explained by an underestimation of the NE content of this feedstuff. As this ingredient is not frequently used as ingredient in commercial sow feed, the amount of energy generated by hindgut fermentation (Schrama and Bakker, 1999) is probably not fully taken into account in current feed evaluation software. As already discussed, RS fermentation will mainly result in butyrate (Martin et al., 1998; van der Meulen et al., 1997) which is known as a precursor for lipogenesis (Rémésy et al., 2004). Potientially, maintenance of RS fed sows could be lower due to reduced activity (Schrama and Bakker, 1999). However, as sows were housed in farrowing crates during the period they consumed the RS diet, the effect of reduced activity was probably small in comparison to group housed gestating sows.

In conclusion, the present study clearly showed that even in the short period of one week before farrowing, dietary fibre type modulates lactation feed intake. The results documented that peripartal diets based on sugar beet pulp had a positive influence on lactational feed intake. However, the physiology behind this phenomenon remains unclear. Furthermore, it was also shown that raw potato

starch can be used as an alternative fibre source for sows to improve peripartal body condition of sows.

Acknowledgement

General Discussion

Brief overview of the study results

Chapter 3. Dose-response effect of fish oil in parturition feed

- ✓ Dose-responsive incorporation of n-3 PUFA in erythrocytes membranes.
- ✓ Linear increased oxidative susceptibility of erythrocytes with increasing fish oil intake.
- ✓ Linear decreased piglet performance with increasing fish oil intake.

Chapter 4. Feed intake regulating hormones in peripartal sows

- ✓ Peripartal profile of leptin, ghrelin, and resistin not affected by feed intake.
- ✓ Only leptin profile was elevated when sows had more back fat at late gestation.

Chapter 5. N,N-Dimethylglycine (DMG) in parturition feed

- ✓ DMG supplementation improved apparent fecal digestibility.

Chapter 6. *Ad libitum* feeding of peripartal sows

- ✓ *Ad libitum* feeding resulted in less mobilization of back fat throughout the peripartal period and throughout lactation.
- ✓ If sows had less than 22 mm back fat at late gestation, *ad libitum* feeding improved piglet performance.

Chapter 7. Functional fibres in parturition feed

- ✓ Sugar beet pulp as fibre source improved voluntary feed intake throughout lactation.
- ✓ Sows fed raw potato starch were more capable of maintaining their back fat reserves throughout the peripartal period.
- ✓ Raw potato starch was most capable of preventing peripartal constipation.

8 General discussion

Modern sow herds often have to cope with problems during the peripartal period, which are almost always detrimental for performance in the current or the subsequent reproduction cycle. Often, these problems are not caused by infectious agents, but are related to suboptimal management or inappropriate nutrition. Hence, optimal feeding of peripartal sows can prevent production losses and in problem cases even resolve the problem. Depending on the particular problem, peripartal feeding strategies can be adjusted and performance can be improved. Even when no specific problems occur, sows will benefit from proper feeding adjusted to the particular metabolic demands of the peripartal period. The importance of peripartal problems such as dystocia, hypophagia, or hypogalactia are widely recognized; however, scientific literature on peripartal feeding strategies remains scarce.

We noticed that the more literature we searched about peripartal problems in sows, the more related to each other they seem to be. Also in practice, we noticed that sows mostly have to deal with a series of problems instead of suffering from a single one. Therefore, we propose to summarize all these performance limiting issues, occurring during the peripartal period, as the sow peripartal syndrome. After identifying the major symptoms of the peripartal syndrome, and discussing possible solutions, theory will be translated into practice. Based on the theory a peripartal scoring system will be set up which can be used in the field to evaluate the peripartal situation in a sow herd.

8.1 The peripartal syndrome

Similar to highly producing dairy cattle, the metabolism of highly prolific sows is seriously challenged around parturition. Several hormones and metabolites change during this short period of time (internal changes) (Ellendorff et al., 1979; Mosnier et al., 2010). In addition, sows also have to cope with external management related changes such as altered housing (from group housing during gestation to individual housing in farrowing crates) (Oliviero et al., 2008), change of feed (from gestation to lactation diet) and feeding strategy (from restricted to *ad libitum*) (Koketsu et al., 1996). Due to the internal changes and the stress caused by the external changes it is not surprising that these high-productive sows are susceptible to metabolic disorders, resulting in production losses.

In Figure 8.1 an overview of the different symptoms included in the sow peripartal syndrome are summarized.
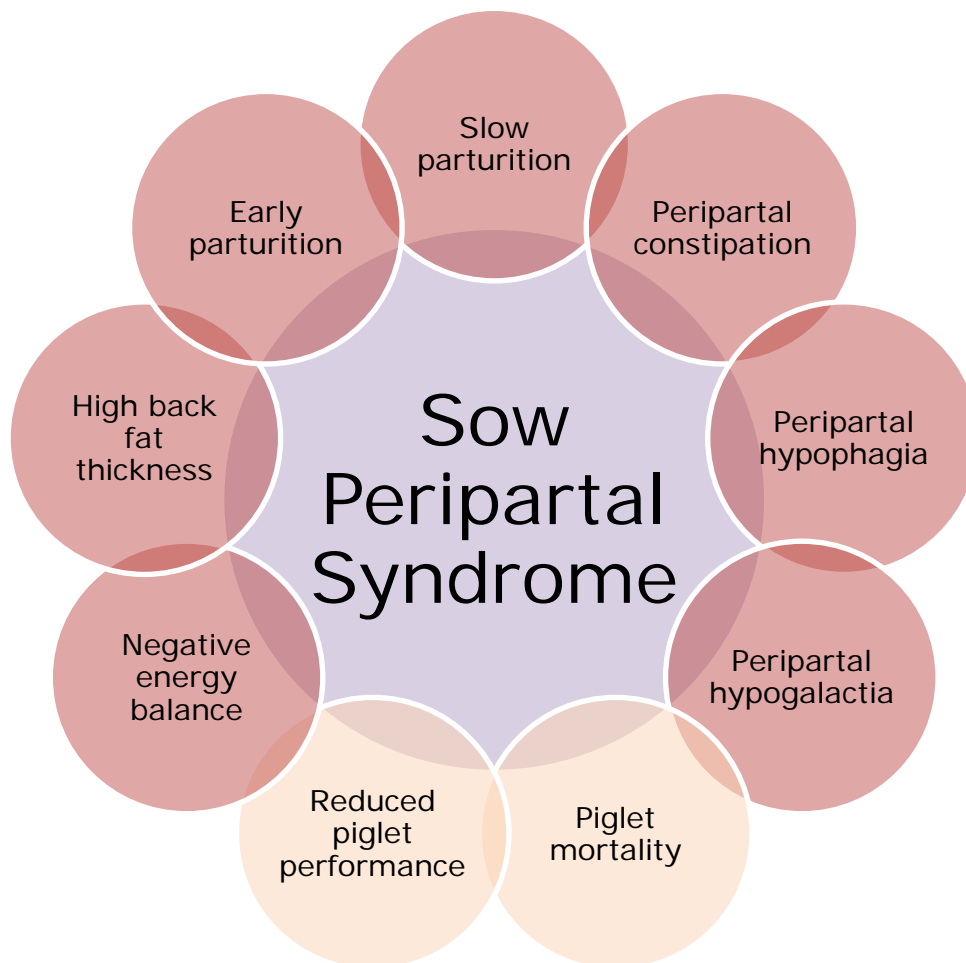

Figure 8.1. The sow peripartal syndrome. Features relating to sows or piglets are indicated in a different color.

8.1.1 Negative energy balance

Throughout gestation, nutrient demands of sows increase exponentially towards parturition. A proper feeding strategy is thus warranted to prevent excessive gain of body condition, in the case of over-feeding, or excessive losses, when intake is not covering the demands (Close and Cole, 1986). Especially during the last month of gestation, metabolic demands of sows are high as a result of the exponential growth of piglets and the development of mammary tissue (Close et al., 1985). It is, therefore, not surprising that sows can become catabolic during the last week of gestation (Le Cozler et al., 1999). Inadequate nutrient intake during the last days prior to parturition can affect piglet birth weight and vitality (Coffey et al., 1994).

Several strategies can be applied to prevent excessive mobilization of body reserves and to keep sows in optimal condition for the approaching lactation. A first strategy can be to improve diet digestibility so that ingested nutrients can be used more efficiently. This can be realized by diet processing (Callan et al., 2007; Wondra et al., 1995) or by adding digestibility enhancing supplements, such as an emulsifier (Dierick and Decuypere, 2004; Jones et al., 1992) or an enzyme (for example phytase (Jongbloed et al., 2000; Liesegang et al., 2005) or lipase (Dierick and Decuypere, 2004)). However, except for studies concerning phytase (Liesegang et al., 2005), all these latter studies were performed on growing pigs. Our research demonstrated that adding N,N-dimethylglycine as an external emulsifier to a parturition diet for sows improved the nutrient digestibility of the diet (**Chapter 5**). Unfortunately, this enhanced digestibility was not translated in improved sow performance. A possible explanation for the lack of effect could be the low number of litters included in the study (n = 13 control and n = 12 supplemented sows) or the relative short period of supplementation (14 days).

A second strategy consists of increasing feed intake during the peripartal period. In the first place, this can be realized by preventing peripartal hypophagia as will be described hereafter (8.1.6). Furthermore, the commonly applied restricted feeding scheme around parturition (Koketsu et al., 1996) can be replaced by an alternative scheme, feeding sows more to appetite to prevent nutrient shortages. Although high feed intake during late gestation was identified as a risk factor for the occurrence of metritis-mastitis-agalactia (MMA) (Göransson, 1989b; Persson

et al., 1989) or during early lactation as a risk factor for peripartal hypogalctia syndrome (PHS) (Papadopoulos et al., 2010), some studies indicated that when *ad libitum* feeding was introduced before parturition the incidence of MMA was lower than when *ad libitum* feeding was applied after parturition (Neil et al., 1996). In a study comparing *ad libitum* feeding during the peripartal period in comparison to a commonly applied restricted feeding scheme, we found that on the premise that sows had no more than 22 mm back fat at late gestation, *ad libitum* feeding was beneficial for both body condition of sows and growth of their litters (**Chapter 6**). Strikingly, hardly any differences between metabolic parameters were detected between the different feeding strategies.

8.1.2 High back fat thickness at late gestation

Research in the early nineties stated that lactational feed intake was negatively correlated with the amount of feed consumed throughout gestation (Dourmad, 1991; Weldon et al., 1994a). Some years later, however, research revealed that the level of body fatness at late gestation was the major determining factor for the lactational voluntary feed intake (Revell et al., 1998b). A possible explanation for the relation between body fatness and feed intake is through leptin. This hormone, mainly secreted by adipocytes, suppresses voluntary feed intake in pigs (Barb et al., 1998). For sows, a strong positive correlation between back fat thickness at parturition and leptin concentration was determined (De Rensis et al., 2005), which might explain why high levels of body fat led to peripartal hypophagia (Revell et al., 1998b). When considering both back fat thickness and amounts of feed offered to sows during the peripartal period, we observed higher leptin levels for fat sows regardless of amount of feed offered (**Chapter 4**). Nevertheless, we could not detect any effect of back fat thickness on voluntary feed intake throughout lactation (**Chapter 6**). This indicates that, although high back fat thickness is a risk factor, it not necessarily leads to severe problems. Likely also other management factors determined whether problems will occur or not.

One could assume that fat sows at late gestation would benefit from their fat reserves during lactation and overcome shortage of energy by mobilization of those reserves. However, milk production of these fatter sows is often lower compared to their leaner counterparts (Revell et al., 1998a). According to Revell et al. (1998a), this is due to better mammary development in lean sows.

Likewise Farmer and Sørensen (2001) reviewed that excessive fat depositon during gestation was detrimental to mammogenesis. We recorded reduced piglet growth for fat sows, but only when fed *ad libitum* during the peripartal period. Although the latter sows consumed similar amounts of feed as leaner sows, they lost more back fat during lactation which was not translated into improved piglet growth (**Chapter 6**, Schenkel et al., 2010). These findings suggest that sows with more fat reserves have higher requirements for maintenance with lower availability to their progeny. In Figure 8.2, we plotted average values of back fat loss during the peripartal period versus net energy (NE) intake during the peripartal period based on the results of **Chapter 3, 6, and 7,** and classified them into the three body condition categories as described in **Chapter 6**. Also the data of Neil (1996) were added to the graph. For the conversion of ME to NE the conversion equation NE (MJ/kg) = 0.78 × ME (MJ/kg) − 1.96, as described by Just (1982), was applied. Sows with less than 22 mm of back fat at late gestation lost less back fat during the peripartal period with increasing NE intake, whereas this was not observed for fat sows with more than 22 mm of back fat. A possible explanation could be that fat sows have higher maintenance requirements compared to its leaner counterparts. Also differences in milk production or efficiency of milk production could explain the absence of response to increasing NE intake.
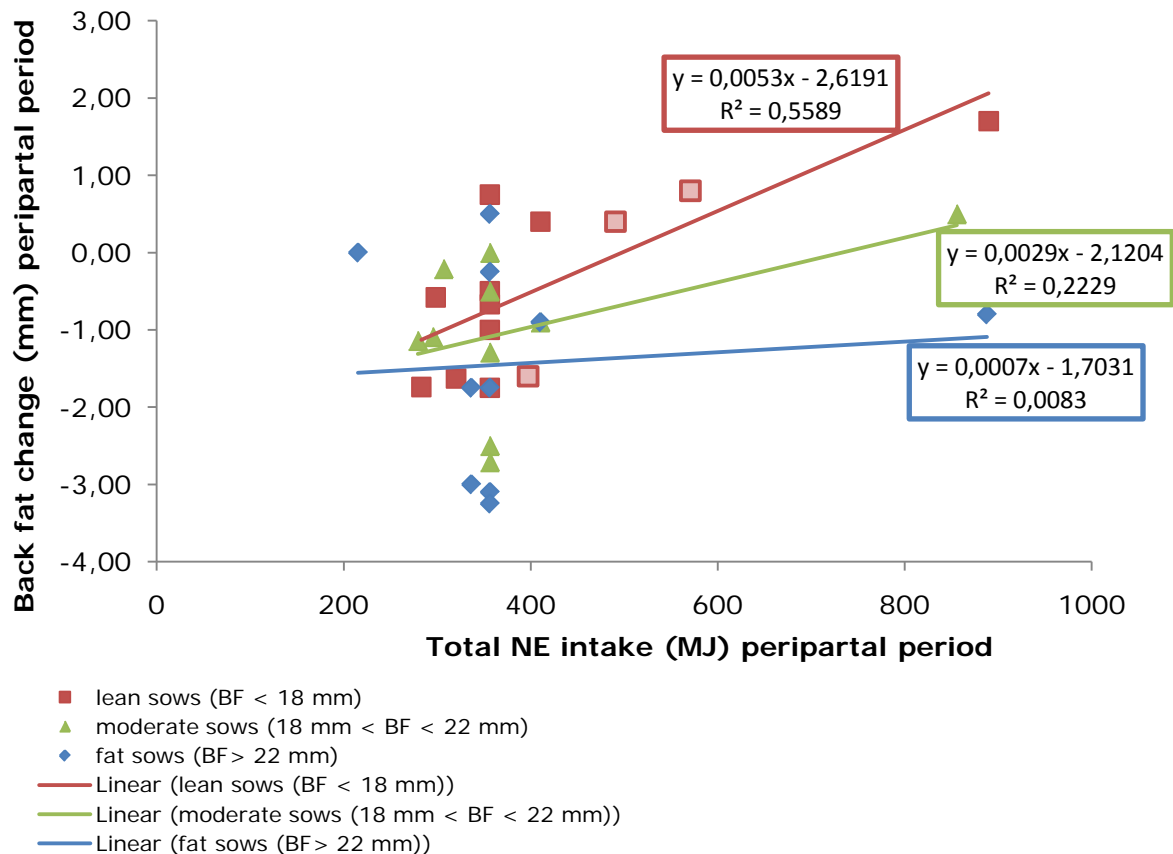
Figure 8.2. Peripartal back fat change versus total peripartal NE intake. Peripartal period was taken between 5 days before and 7 days after parturition. Negative back fat change represents loss of back fat whereas positive values indicate gain of back fat. Full colored symbols represent data (each point is the average of one treatment group) obtained in **Chapter 3, 6 and 7** whereas light colored symbols represent data from literature (Neil, 1996).

In order to avoid problems, it is, therefore, recommended to adjust sows gestational feeding schemes to their needs and prevent overconditioning at the moment they are moved to the farrowing room.

8.1.3 Early parturition

Normal gestation of sows lasts three months, three weeks, and three days, or approximately 115 days (Cowart, 2007). In current practice, 80 % of sows have a gestation length between 114 and 117 days, whereas early parturition occurs in 10 % of all farrowings (Sasaki and Koketsu, 2007; Vanderhaeghe et al., 2011). Large litter size is suggested as an important cause for early parturition (Rydhmer et al., 2008; Sasaki and Koketsu, 2007). This is not surprising given that in sows parturition is initiated by increased cortisol production of fetuses as a result of stress caused by uterine crowding (Davidson and Stabenfeldt, 2007; Devillers et al., 2006). Fetal cortisol subsequently results in increased secretion

of placental estrogen and endometrial prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$), which on their turn initiate the actual expulsion of piglets (Cowart, 2007; Davidson and Stabenfeldt, 2007). Besides the effect of litter size, breed was also identified as a determinant of early parturition (Rydhmer et al., 2008). Risk for preterm parturition increased when sows farrowed early during the preceding cycle, indicating a high repeatability of gestation length (Sasaki and Koketsu, 2007; Vanderhaeghe et al., 2011). A more thorough study identifying different risk factors for early parturition is, to our knowledge, not published yet, but it can be assumed that besides litter size and breed, other management related factors such as energy balance or feeding strategy could affect gestation length. Furthermore, infections with pathogens such as porcine reproductive and respiratory syndrome (PRRS) virus or parvovirus are also very important (Maes et al., 2007), but outside the scope of this thesis.

As a result of a shortened gestation length, piglets will be less developed at parturition resulting in more stillborn piglets (Sasaki and Koketsu, 2007; Vanderhaeghe et al., 2011) and impaired preweaning performance as piglets with low birth weight and vitality will ingest less colostrum (Devillers et al., 2007; Rydhmer et al., 2008). Moreover, when parturition in sows was induced before day 112 of gestation, colostrum contained significantly less energy (Jackson et al., 1995). Recently, it was shown that colostrum quantity correlated positively with gestation length (Decaluwé et al., 2013). Therefore, it is recommended to avoid parturition before 114 days of gestation.

Preventive measures described in literature include hormonal treatment affecting the progesterone-prostaglandin metabolism (Foisnet et al., 2010a; Vanderhaeghe et al., 2011). Alternatively, feed during late gestation can be supplemented with n-3 poly unsaturated fatty acids (PUFA) to lower the synthesis of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$). As visualized in Figure 8.3, adding fish oil, as a source of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), increase the production of prostaglandins (PG) of the 3 series at the expense of PG of the 2 series as they compete for the same enzyme (Wathes et al., 2007). These PG of the 3 series are biologically less active and less capable of inducing myometrial contractions (Abayasekara and Wathes, 1999; Allen and Harris, 2001; Wathes et al., 2007) and luteolyis (Abayasekara and Wathes, 1999). On the contrary, augmentation of n-6 PUFA in the diet can induce preterm parturition (Wathes et al., 2007). By adding fish oil to the diet of gestating sows,

gestation length can be prolonged by 0.5 days (Rooke et al., 2001a). However, in our dose-response study (**Chapter 3**), no effect of different dosages of fish oil on gestation length was recorded, but it should be mentioned that on this farm no problems with early parturition were reported. On the contrary, in **Chapter 6** we observed early parturition (average gestation length 113.2 ± 1.2 days) although litter size was not extremely large. Taking a closer look at the diet offered to the sows during late gestation, the main sources of fatty acids were rapeseed cake (3 %) and soy bean oil (2.1 %). Both ingredients are rich in linoleic acid (n-6 PUFA) which could partly be responsible for the preterm parturition on that study farm. This confirms research in ewes indicating that n-6 PUFA could stimulate early parturition (Elmes et al., 2005) but needs further research in sows.
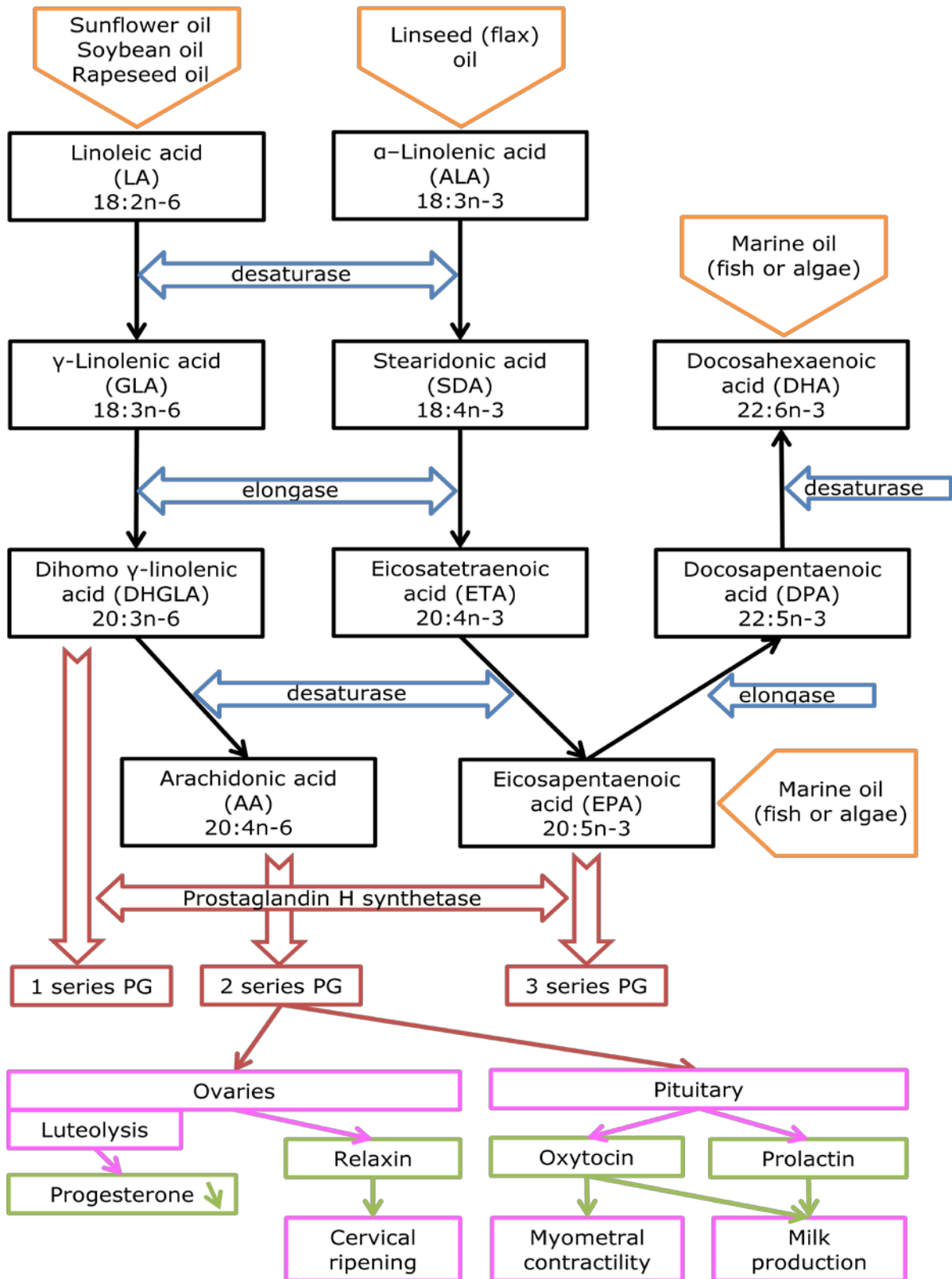
Figure 8.3. Effect of dietary oils on the parturition process in sows. Black boxes represent fatty acids, orange boxes dietary oil, red boxes prostaglandins (PG), pink boxes reproduction related organs, and green boxes represent hormones, fatty acid synthesis related enzymes are presented in blue arrows and those related to PG synthesis in red arrows. (after Abayasekara and Wathes, 1999; Allen and Harris, 2001; Davidson and Stabenfeldt, 2007; Wathes et al., 2007).

8.1.4 Slow parturition

Parturition in sows is a stressful event which is associated with elevated cortisol levels. As a result of stress during parturition, depressed oxytocine levels could be observed leading to prolonged parturition (Lawrence et al., 1992). Several reasons for this stress were previously described, namely pain related to the parturition itself (Mainau and Manteca, 2011), environmental stress related to the altered housing circumstances (Lawrence et al., 1995), restraining sows in farrowing crates (Oliviero et al., 2008), discomfort as a result of peripartal constipation (Oliviero et al., 2009), or reduction of feed offered as often practiced prior to parturition (Papadopoulos et al., 2010; Persson et al., 1989). Furthermore, Oliviero et al. (2010) identified increased back fat thickness at late gestation as a factor prolonging parturition via affected oxytocine receptor activation. Our studies did not reveal any effect of body condition or peripartal feed intake on duration of the parturition process (**Chapter 6**). Although several researchers postulated that farrowing duration is influenced by litter size (Zaleski and Hacker, 1993), van Dijk et al. (2005) reported an inverse relation between gestation length and duration of parturition, regardless of litter size. In order to avoid the discussion about what the exact duration of parturition should be, it is better to time the interval between two subsequent piglets born. If this interval does not exceed 30 min, their are no problems with parturition progress (Le Cozler et al., 2002).

As a result of prolonged parturition, piglets can suffer from asphyxia during the actual delivery (Herpin et al., 1996). This can increase the number of stillborn piglets (Canario et al., 2006) or affect the piglets' vitality, which consequently can lead to decreased piglet growth and increased preweaning mortality (Herpin et al., 1996). However, farmers can intervene and apply birth assistance in order to prevent piglet losses due to asphyxia. Apart from the time-consuming and labour-intensive character of birth assistance, it also increases the risk of developing MMA (Gerjets et al., 2011), resulting in further weakening of the piglets (Kemper and Gerjets, 2009), and even increase mortality (Hirsch et al., 2003). Furthermore, indications of increased incidence of peripartal hypophagia with prolonged parturition were reported (Tummaruk and Sang-Gassanee, 2013) which again results in reduced milk production and piglet performance.

A proper solution for the problem of slow parturition is, to our knowledge not yet described, but could consist of solving the identified causes such as high body condition at late gestation (8.1.2), peripartal constipation (8.1.5), or early parturition (8.1.3).

8.1.5 Peripartal constipation

Constipation was frequently reported in peripartal sows (Chapter 4, Tabeling et al., 2003). Research in humans has pointed out that there is a close relationship between reproductive hormones and functionality of the enteric nerve system (Mathias and Clench, 1998). Mainly progesteron and estrogen, both high during late gestation in sows (Ellendorff et al., 1979), prolonged gastric emptying (Bonapace and Fisher, 1998) and decreased colonic muscle contractility (Bonapace and Fisher, 1998; Cullen and O'Donoghue, 2007; Wald, 2003), resulting in gastrointestinal hypomotility. Hence, this lowered passage rate can result in constipation (Bonapace and Fisher, 1998; Cullen and O'Donoghue, 2007; Wald, 2003). In addition to the reduced gastrointestinal motility, progesteron and estrogen also increased colonic water absorption via increased renin production which aggravated the incidence of constipation (Cullen and O'Donoghue, 2007). Besides this high hormonal predisposition for the occurrence of constipation, some commonly applied management measures during late gestation can increase the risk for constipation. As already mentioned (8.1.1), restricted feeding of sows during late gestation is more or less standard in most sow herds. By decreasing the amount of feed ingested, stomach emptying is reduced which subsequently lowers passage rate (Black et al., 2009). Tabeling et al. (2003) reported a lower defaecation frequency for restricted fed peripartal sows in comparison with *ad libitum* fed sows. Furthermore, the latter study also reported drier feces when feeding sows restrictedly. Parallel with decreased amounts of feed offered, sows were often fed a lactation diet low in fibres from some days before parturition. This lowered fibre ingestion mostly results in drier feces (Tabeling et al., 2003) and increased incidence of severe constipation (Oliviero et al., 2009).

Constipation resulting in the presence of hard fecal material in the colon and rectum of the sow can impede the passage of the piglet through the birth canal during parturition and increase the risk for dystocia (Cowart, 2007). Besides the physical barrier the feces is forming for the piglet, it is an uncomfortable and

painful feeling for the sow. This stress can lead to elevated opoid production which on its turn inhibits oxytocin function and prolongs parturition (Oliviero et al., 2010). It is, therefore, not surprising that peripartal constipation is negatively correlated with farrowing duration (Oliviero et al., 2010) and can increase the number of stillborn piglets. When passage rate of the digesta slows down, intestinal microbiota get more time to proliferate and secretion of endotoxins increases (Tabeling et al., 2003). Absorption of these endotoxins can result in MMA (Tabeling et al., 2003) or in less severe cases to PHS (Oliviero et al., 2009). Therefore, peripartal constipation was identified as a risk factor for MMA (Hermansson et al., 1978) or PHS (Maes et al., 2010). Hence, it is of no surprise that offering sows straw (Göransson, 1989b) or feeding them a bulky diet (Göransson, 1989a) during late gestation resulted in a decreased incidence of PHS. Likewise, studies investigating the effect of bulky diets during late gestation often reported improved piglet growth during the first week of lactation for the sows fed the bulky diet (Guillemet et al., 2007; Oliviero et al., 2009). The question, however, remains which type of fibre should be added to the peripartal diet. Whereas most studies investigated the effect of control versus high fibre diets using wheat bran (Meunier-Salaun et al., 2001), or a mixture of different fibre sources (Oliviero et al., 2009; Tabeling et al., 2003), we compared different types of fibres in parturition diets (**Chapter 7**). Although most sows only suffered from mild constipation (less than 3 days no feces production), the group of sows fed the diet containing 15 % of raw potato starch has the least affected sows. Although on a chemical basis, this ingredient is not an actual fibre, it was able to improve passage rate and prevent peripartal constipation.

Peripartal constipation can be avoided by feeding sows adequate amounts of a fermentable feed during the peripartal period. Furthermore, it remains important that sows get *ad libitum* access to fresh drinking water of sufficient quality.

8.1.6 Peripartal hypophagia

Decreased voluntary feed intake or even anorexia during early lactation is commonly reported in highly prolific sows (Knap, 2009). Already during the nineties, Koketsu et al. (1996) reported in a large screening of 25,000 sows on 30 herds, that 32.9 % of sows suffered a major drop and 27.8 % a minor drop in feed intake during early lactation. This hypophagia has been associated with the occurrence of hypogalactia (Papadopoulos et al., 2010), reduced litter

performance (Koketsu et al., 1997), increased mobilization of body reserves (Revell et al., 1998b), prolonged weaning to oestrus interval (Dourmad et al., 1994), and even reduced reproductive performance during the subsequent cycle (Cromwell et al., 1989). It is, therefore, not surprising that sows with inadequate lactational feed intake were more likely to be culled early (Anil et al., 2006). Selection towards a leaner type of pig with better lean feed conversion could be at the basis of this reduced voluntary feed intake of lactating sows (Bergsma et al., 2009; Gilbert et al., 2012; Kerr and Cameron, 1996). Also body condition of sows at the moment of farrowing is of great importance. Several authors reported a negative correlation between back fat thickness at late gestation and lactational feed intake (Mullan and Williams, 1989; Revell et al., 1998b). A possible explanation for the relation between the amount of back fat and voluntary feed intake could be through leptin. Leptin is a key metabolic signal to the brain reflecting energy stores and energy balance (Summer et al., 2009) and is mainly secreted by adipose tissue (Barb et al., 2001). As already discussed (8.1.2), sow leptin levels are positively correlated with back fat depth around parturition (**Chapter 5**, De Rensis et al., 2005; Estienne et al., 2003). For lactating primiparous sows a positive correlation between leptin levels and back fat thickness as well as a positive correlation between leptin and energy intake was reported (Prunier et al., 2001). However, in multiparous sows, we could not detect any correlation between feed intake and leptin levels (**Chapter 4**). Despite the positive relation between back fat and leptin (**Chapter 4**) high back fat levels at late gestation were not detrimental for lactational feed intake (**Chapter 6**). This indicates that leptin is probably not the only factor affecting voluntary feed intake throughout lactation. Our findings that peripartal leptin levels were not related to sows feed intake (**Chapter 4**), support this hypothesis.
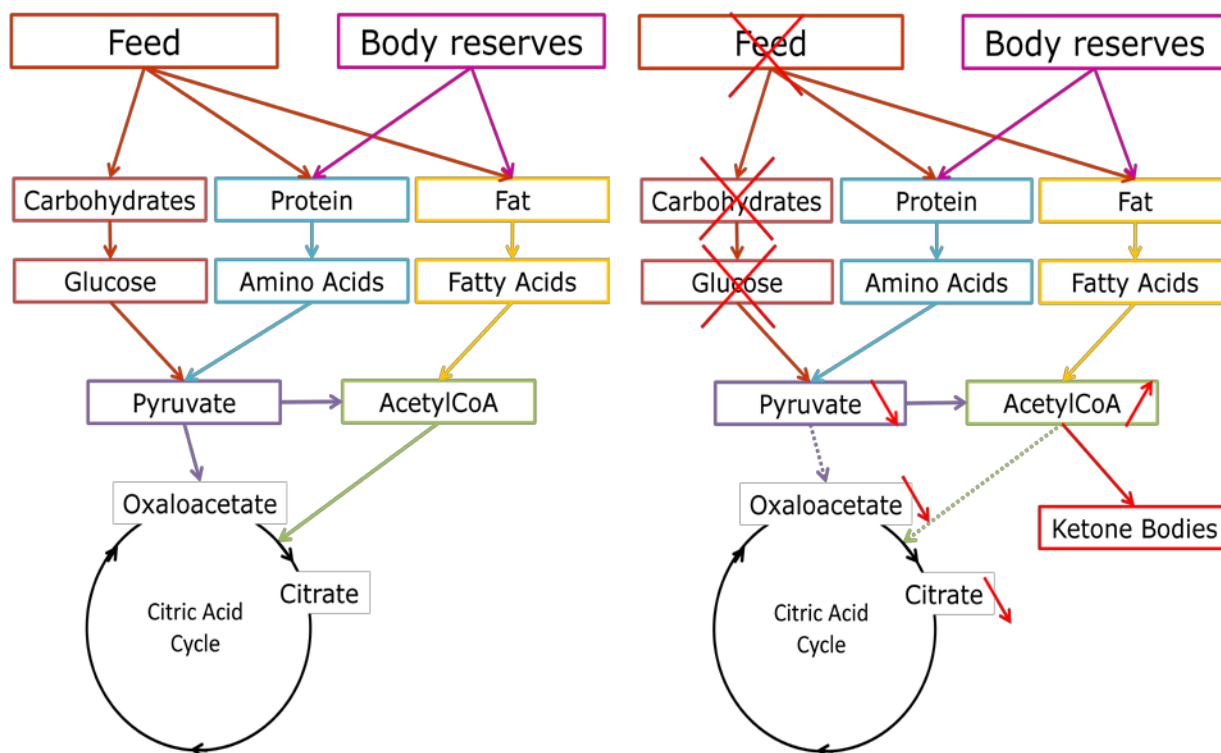
Figure 8.4. Overview of energy metabolism through the citric acid cycle when the amount of acetylCoA and oxaloacetate is balanced (left), or through ketone production when amount of puryvate is reduced as a result of hypophagia or anorexia which resulted in unbalanced amounts of acetylCoA and oxaloacetate (right).

There is evidence that not only the amount of back fat but the combination of high fat and low protein reserves are detrimental for lactational feed intake (Sinclair et al., 2001). When high fat reserves are compensated by sufficient protein reserves, there is no influence of lactational feed intake. A possible hypothesis for this observation could be that when high amounts of protein are present they could be used as alternative source for pyruvate synthesis. This prevents that the citric acid cycle gets blocked and the metabolism shifts to less efficient energy production through ketone bodies (Figure 8.4). These ketone bodies are known to cause nausea which will further decrease voluntary feed intake (Alsop et al., 1994).

Although the sow herself is an important factor, several management related factors such as housing, feeding strategy and feed composition are of great importance. From the moment sows start farrowing, ambient temperature in the farrowing room rises due to switching on infrared lamps and floor heated piglet nests. Mostly temperature rises above 22 °C, the upper critical temperature of the thermoneutral zone of the lactating sow (Black et al., 1993), which can impair voluntary feed intake (Christon et al., 1999; Messias de Bragança and

Prunier, 1999). Composition of the diet can play an important role in this. By adding 6 % of fat to the lactation diet, energy intake of lactating sows under tropical conditions could be increased by approximately 25 % during the first week of lactation (Christon et al., 1999). Also when comparing lactation diets high in fat versus diets high in starch, sows on the high starch diet consume more feed but produce more heat and have a lower milk energy output (van den Brand et al., 2000). This makes sows on a high starch lactation diet in a hot environment less efficient and susceptible to possible feed intake problems. However, high levels of dietary fat around parturition can have a negative effect on lactational feed intake via the glucose metabolism. By feeding sows high levels of energy during gestation, they become more glucose intolerant and more insulin resistent during lactation (Weldon et al., 1994b; Xue et al., 1997). This reduced glucose clearance could lead to reduced feed intake. Furthermore, it is described that additional fat during the last month of gestation resulted in glucose intolerance where this was not the case when using additional starch as energy source during this same period of time (van der Peet-Schwering et al., 2004). However, this latter study did not observe differences in feed intake during the first two weeks of lactation between the different diets. When comparing a lactation diet high in fat versus a lactation diet high in starch, the sows on the high fat diet catabolized more protein reserves compared to those in the high starch diet (Nelssen et al., 1985b). More recently, Theil et al. (2012) succeeded to induce ketosis in sows using high fat diets (10 % of crude fat). These findings are in accordance with the earlier stated hypothesis that by shortage of oxaloacetate precursors and an excess of fatty acids, either from body fat mobilization or from increased dietary fat intake, the energy metabolism is shifting towards the ketogenic side (Figure 8.4).

Sifting out literature comparing feeding strategies applied during the first week of lactation, a gradually increasing feeding scheme is often applied (Kruse et al., 2011; Mosnier et al., 2010) in order to prevent a drop in lactational feed intake (Koketsu et al., 1996). Other studies, however, shifted sows from restricted feeding towards *ad libitum* feeding one day after parturition (Guillemet et al., 2006; Quesnel et al., 2009; Trottier and Easter, 1995) which resulted in a drop of feed intake one day later. The alternative of introducing *ad libitum* feeding from before parturition is, however, rarely practiced. Our findings (**Chapter 6**) on this feeding strategy are in accordance with those of Neil (1996) and pointed

out that this can be a useful alternative to keep lactational feed intake as high as possible.

The question, however, remains why results are ambiguous. Probably the combination of body condition and applied feeding strategy is of great importance. Whereas in the early nineties reports stated that high gestational feed intake resulted in lowered lactational feed intake (Weldon et al., 1994a), more recent research reported that gestational feeding strategy is only a measure to assure appropriate body condition at the moment of farrowing (Young et al., 2004). By entering sows into the farrowing room in optimal body condition, feed intake problems can be prevented. Especially when sows have high amounts of fat reserves which are not balanced by appropriate amounts of protein reserves, intake of lactation diets with mainly fat as energy source can be problematic.

Therefore, preventive measures against peripartal hypophagia include maintaining optimal body condition of sows and balanced energy sources (fat versus carbohydrates) in the peripartal and lactation diet. Furthermore, feeding a bulky gestation (Farmer et al., 1996; Guillemet et al., 2006; Quesnel et al., 2009) or peripartal diet (**Chapter 7**) can improve lactational feed intake. Mainly sugar beet pulp turned out to be beneficial in order to promote lactational feed intake (**Chapter 7**). Also the transition to the lactation diet was easier (Guillemet et al., 2010), and incidence of constipation (Oliviero et al., 2009) and agalactia (Göransson, 1989a) were lower when feeding a fibrous diet during the peripartal period. Finally, *ad libitum* feeding introduced from before parturition, in stead of shortly after parturition, can prevent a drop in feed intake during lactation (**Chapter 6**, Neil, 1996) on the premise that sows are in optimal body condition and the appropriate feed is used.

8.1.7 Peripartal hypogalactic syndrome

Sows with peripartal hypogalactic syndrome are present in approximately one out of three sow herds in Flanders (Papadopoulos et al., 2010). Affected sows very often do not show clinical signs of inflammation like fever, pain, or swollen udders, but their piglets seem to be hungry, have a higher risk to die, and by the end of the first week of lactation litters become heterogenous (Papadopoulos et al., 2010). Although the economic importance of PHS is clear, not much about the pathophysiology of this syndrome is known yet (Maes et al., 2010). Research

has pointed out a heritibility of 0.10 for PHS indicating a genetic predisposition (Preissler et al., 2012). However, more management related predisposing factors like feeding, body condition of sows, peripartal constipation, and hygiene are probably of greater importance.

In recent studies, a negative correlation between progesterone concentrations around parturition and piglet growth during the first week of lactation is observed (de Passille et al., 1993; Quesnel et al., 2013). Also sows with low colostrum production appear to have higher progesteron levels than high yielding sows (Foisnet et al., 2010b). These findings indicate a possible role of importance of progesteron levels in PHS affected sows. They also can explain why earlier research reported positive effects on litter growth when using $PGF_{2\alpha}$ treatment shortly after parturition (Liptrap, 1980; Morrow et al., 1996) and why induction of parturition by means of $PGF_{2\alpha}$ was adviced as preventive measure against MMA (Einarsson et al., 1975). By decreasing the synthesis of $PGF_{2\alpha}$ at late gestation in order to avoid early parturition, normal luteolysis could be impaired and milk production afterwards could be lowered. By adding fish oil, as a source of n-3 fatty acids, to the diet, production of progesterone by the corpa lutea can be increased (Abayasekara and Wathes, 1999). This resulted not only in prolonged gestation (Rooke et al., 2001a) like already described (8.1.3) but also delayed luteolysis (Wathes et al., 2007). Considering this, the linear increased piglet mortality and reduced litter weight gain with increasing concentrations of fish oil in the peripartal diet of sows, as observed in **Chapter 3**, could be the result of a subclinical PHS that was not diagnosed at that time. In accordance with our findings, lower milk (Laws et al., 2009) and milk energy output (Lauridsen and Danielsen, 2004) accompagnied by reduced litter weight gain for sows supplemented with fish oil during late gestation were previously reported.

Progesteron is a lipophilic steroid hormone that can be stored in body fat. Therefore, sows with high amounts of back fat may have a delayed decline of progesteron levels during the first 48 h postpartum (Miller et al., 2004; Oliviero et al., 2010). The identification of high back fat thickness as a risk factor for the occurrence of PHS (Maes et al., 2010) confirmed this hypothesis. Also catabolism of body fat around farrowing can prevent a rapid progesteron decline postpartum, as again these fat reserves can be a source of progesteron. However, this can explain why highly prolific sows, which are often catabolic around parturition due to their high metabolic demands, are more likely to

develop PHS. Likewise, sows with hypophagia or even anorexia around parturition, mobilizing significant amounts of body fat, could be more susceptible for PHS. This confirms the results of Papadopoulos et al. (2010) who reported that 62% of all herds in Flanders affected with PHS also suffered peripartal hypophagia. In contrast with this, the latter study of Papadopoulos et al. (2010) identified *ad libitum* feeding during early lactation to increase the risk for PHS, confirming earlier results (Neil et al., 1996). This seems contradictory with our earlier hypothesis, but as we discussed before (8.1.5), sows that were introduced to *ad libitum* feeding immediately after parturition often showed a significant drop in feed intake the day afterwards. By introducing *ad libitum* feeding before farrowing, this feed intake drop can be avoided (**Chapter 6**) and sows are less prone to PHS (Neil et al., 1996).

Next to an alternative storage of progesteron, high amounts of body fat in sows can lead to chronic inflammation (Spurlock and Gabler, 2008). Furthermore, Sauber et al. (1999) reported decreased milk production as a result of inhibition of lactogenic hormones by proinflammatory cytokines. As for both humans (Norman et al., 2007) and cows (Koets et al., 1998) parturition may result in release of proinflammatory cytokines, it is probably that this is also the case in sows and explain the link between fatness and occurrence of PHS. Hence, the link between PHS and risk factors like dystocia (Backstrom et al., 1984) or slow parturition (Tummaruk and Sang-Gassanee, 2013), both resulting in birth intervention (Gerjets et al., 2011), low hygenic standards (Hulten et al., 2004), or constipation (Hermansson et al., 1978), all could lead to increased inflammation. Finally, management related risk factors causing stress, like for example transfer to farrowing unit shortly before farrowing (Papadopoulos et al., 2010) or housing system (Backstrom et al., 1984), increased cortisol levels which were related with MMA (van Gelder and Bilkei, 2005).

Preventive measures against PHS are in first place avoiding previously described risk factors. So when sows are at optimal body condition upon entry in the farrowing unit and when they receive a fibre rich diet fed without severe feed intake restriction (**Chapter 7**) the occurrence of PHS could be reduced and piglet performance improved (Guillemet et al., 2007).

8.1.8 Piglet mortality

Piglet mortality was defined by Knol et al. (2002) as the sum of stillborn and preweaning mortality giving an indirect indication of farrowing and preweaning survival, respectively. Stillborn piglets mainly died during parturition (Glastonbury, 1977) and most of them (80 %) are born during the second half of the parturition process (Le Cozler et al., 2002). As already mentioned earlier (8.1.3), stillborn piglets most likely suffered from asphyxia during the parturition process (Canario et al., 2006; Herpin et al., 1996). Although some infectious diseases can cause stillbirths, mostly this was not the case and their occurrence was related to sow or management factors. In general, stillborn piglets occurred in one out of two litters born, but it is only considered problematic when more than one piglet per litter is stillborn (Vanderhaeghe et al., 2010a).

Preweaning mortality is calculated as the percentage of live born piglets that died before weaning. For preweaning mortality, different infectious and non-infectious causes were identified. However, in general mortality during early lactation is mostly related to management factors whereas mortality after the first 4 days of lactation is most likely the result of infections with pathogens (Vaillancourt and Tubbs, 1992). More than half of the preweaning mortality occurs during early lactation (Marchant et al., 2000; Vaillancourt and Tubbs, 1992) mainly due to crushing or starvation (Edwards, 2002; Hellbrugge et al., 2008; Marchant et al., 2000). Moreover, the risk of piglets being crushed by the sow increased when piglets get more hungry and continuously try to suckle (Hellbrugge et al., 2008; Weary et al., 1996b). Unfortunately, 70 % of crushed piglets were healthy (Vaillancourt and Tubbs, 1992).

Surveys considering reproduction results of Flemish sow herds reported an average number of stillborn piglets between 7.5 and 8.5 %, and preweaning mortality of 10.5 to 14.2 % (Vanderhaeghe et al., 2010b; Vanderhaeghe et al., 2010a; Verheyen et al., 2007). These figures are consistent with those reported in literature (Alonso-Spilsbury et al., 2007; Edwards, 2002; Vaillancourt and Tubbs, 1992). Considering our data from **Chapter 3 to 7**, we had an average of 5.2 % of stillborn piglets and a preweaning mortality of the live born piglets of 11.3 %. This indicated that reproductive performance in our trials was not deviating from commonly reported results.

Intensive genetic selection for increased litter size is often mentioned as primary reason for increasing piglet mortality in larger litters (Chen et al., 2010; Hellbrugge et al., 2008). When plotting all our piglet mortality data from **Chapter 3 to 7** in function of litter size (counted as total born piglets), also an increased percentage of stillborn and preweaning mortality with increasing litter size was observed (Figure 8.5). In contrast with these findings, Milligan et al. (2001) reported that it was mainly the variation in birth weight that determines preweaning survival and not littersize or birth weight as such. Furthermore, some authors reported that certain breeds had higher stillbirth rates (Canario et al., 2006; Vanderhaeghe et al., 2010b). It remains to be elucidated whether this breed effect is partly caused by a higher litter size specific for a breed. Taking a look at our results in Figure 8.5 again, we see that some breeds have more total born piglets than others and subsequently more stillborn piglets. Genetic selection for piglet mortality, however, turned out to be much more difficult due to its low heritability (Hellbrugge et al., 2008; Knol et al., 2002). Besides genotype, also parity is a determinant factor in the number of stillborn piglets. With increasing parity, the incidence of stillbirths within a litter increased (Canario et al., 2006). Moreover, sows that already produced a litter with more than one stillborn piglet had 2.5 times more risk for stillborn piglets during the subsequent cycle (Vanderhaeghe et al., 2010a).

Other factors responsible for piglet mortality can be identified as those resulting in slow parturition. Fraser et al. (1997) reported an average birth interval of 13 min before a live birth and 34 min before stillbirth indicating that all factors prolonging parturition such as back fat thickness at late gestation or peripartal constipation, consequently increased the incidence of stillbirth. Back fat thickness at parturition was described as an important factor affecting the incidence of stillborn piglets. In a large field trial including 22 sow herds, Vanderhaeghe et al. (2010a) found that sows with less than 16 mm back fat at parturition had 2.5 times more risk for stillborn piglets. In line with the latter study, we observed an increased percentage of stillborn piglets for sows having less than 18 mm of back fat (**Chapter 6**). But also when back fat thickness at late gestation exceeded 22 mm more stillbirths were recorded, which is in line with previous findings in primiparous sows (Revell et al., 1998a). Although we did not detect differences in farrowing duration between different body condition groups, we did not measure individual time intervals between individual piglets but calculated the

average birth interval (**Chapter 6**). Also in the two other studies cited, no individual birth intervals were recorded. Yet, it is difficult to conclude wether body condition only affected stillbirth via prolonging parturition or via an alternative pathway. Another possible explanation is that sows with low back fat reserves at late gestation lack sufficient energy for optimal fetal development (Close and Cole, 1986). As a result piglets were weaker and less developed and probably more prone to die just before or during parturition. For sows with high levels of back fat at late gestation, one could assume that there is plenty of energy present for optimal fetal developement. However, we already discussed (8.1.2) that these fat sows have higher maintenance requirements than their leaner counterparts leaving less energy remains for fetal development. Also a higher risk for PHS was observed (8.1.6) which could lead to further weakening of piglets and a higher percentage of preweaning mortaltiy. Besides back fat thickness (8.1.2), also peripartal constipation (8.1.5) is related to prolonged parturition (Oliviero et al., 2010) and identified as an important risk factor for the occurrence of PHS (Maes et al., 2010). It is thus of no surprise that peripartal constipation increased piglet mortality.

Given the close link between slow parturition and stillbirth, and the link between PHS and preweaning mortality, measures preventing slow parturition and PHS are, therefore, also reported as preventive measures for piglet mortality. Hence, prevention of slow parturition can reduce preweaning mortality. Leenhouwers et al. (1999) reported that piglets born in litters with stillborn piglets had lower vitality and were, therefore, more prone to die before weaning. In accordance with these latter findings, reduced gestation length which often resulted in immature piglets at birth, can also result in increased number of stillborn piglets and preweaning mortality (Canario et al., 2006; Rydhmer et al., 2008; Zaleski and Hacker, 1993).
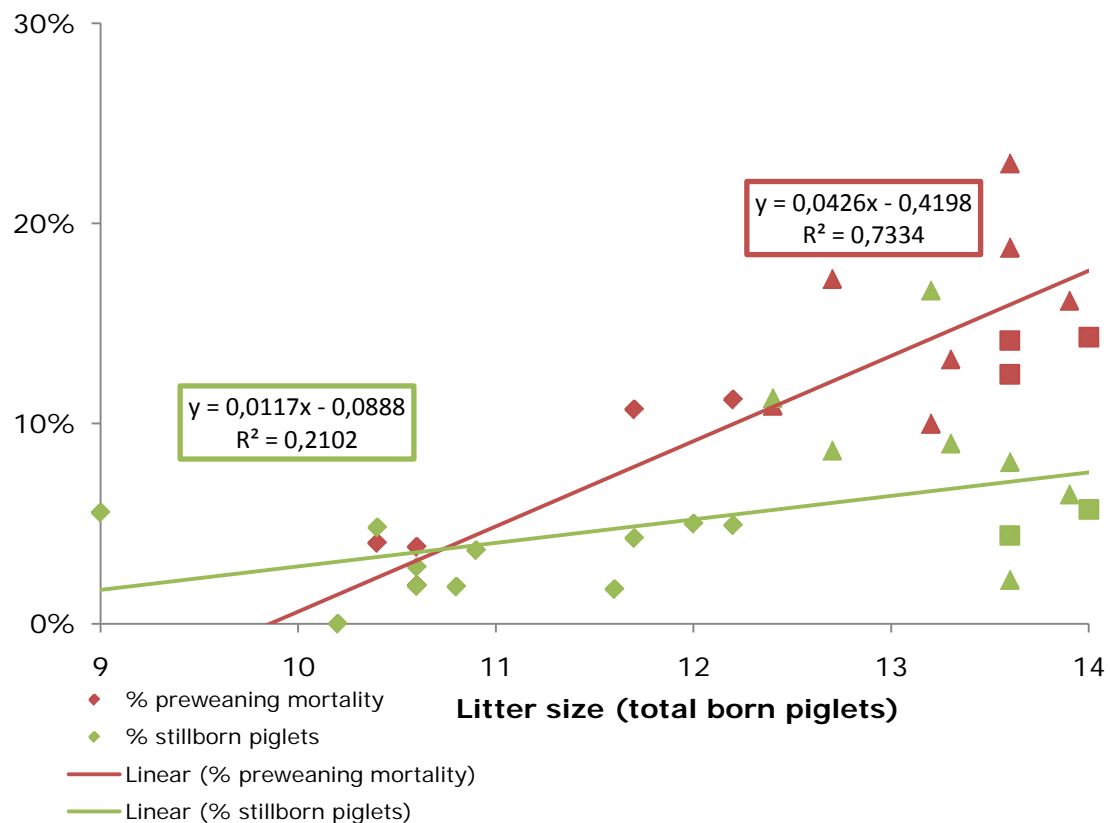
Figure 8.5. Piglet mortality (% stillborn, and % preweaning mortality) of all trials described in chapter 3 to 7 in function of total litter size. Diamonds (♦) indicate the Naima hybrid sows, triangles (▲) indicate the Rattlerow-Seghers hybrid sows and the squares (■) indicate the Landrace crossbred sows.

8.1.9 Reduced piglet performance

In general, glycogen reserves of neonatal piglets decline rapidly after birth (Elliot and Lodge, 1977) making them susceptible to hypothermia. Therefore, fast intake of sufficient amounts of colostrum is of great importance for piglet performance. Based on a review of Quesnel et al. (2012), a colostrum intake of 200 g within the first 24 h after birth seemed to cover the necessary energy demands for survival. However, the higher the colostrum intake the better the preweaning performance of the piglets (Devillers et al., 2007). As birth weight, piglet vitality, and homogeneity of the litter are identified as important factors determining the amount of colostrum consumed (Devillers et al., 2007), dietary and management interventions affecting these latter factors will also affect piglet performance. Piglets with a higher birth weight not only consume more colostrum (Devillers et al., 2007) but are also more vital (Canario et al., 2006) and gain more weight (Milligan et al., 2001) in comparison to lighter piglets. For several decades, research on the effect of gestational diet composition has

focused on increasing energy intake during the last third of gestation aiming to increase litter birth weight. However, only a few studies were successful (Coffey et al., 1994; Papadopoulos et al., 2009b). Most of them did not observe any differences in litter weight at birth (Clowes et al., 2003; Coffey et al., 1987; Quiniou et al., 2008; Seerley et al., 1974). Probably increased energy intake during the last month of gestation only prevents piglets from being underweighted at birth. It is, therefore, probably of greater importance to improve piglet vitality. Although this parameter is difficult to determine, functional ingredients like for example n-3 PUFA can improve vitality (Edwards, 2002; Rooke et al., 2001a). This improved vitality is the result of the enrichement of piglet tissue *in utero* with DHA (Allen and Harris, 2001; Li et al., 2009a; Rooke et al., 2001b) resulting in better organ maturation (Innis, 2005) and improved brain development (Innis, 2007). Moreover, also piglet immunity (Bassaganya-Riera et al., 2007; Wallace et al., 2001) and glycogen reserves (Gabler et al., 2007) could be improved by supplementing DHA to the sows diet. On the contrary, when supplementing different dosages of fish oil during the peripartal period, we did not find beneficial effects on piglet performance (**Chapter 3**).

Another strategy to optimize preweaning piglet performance is by enhancing milk production. As already mentioned, impaired milk production reduced daily weight gain of piglets (8.1.6). Besides prevention for PHS both milk quality and quantity can be influenced by nutritional management of sows. When offering sows a fibrous gestation or peripartal diet, their voluntary lactational feed intake is maximized which is translated into improved liter weight gain (Guillemet et al., 2007; Quesnel et al., 2009). Similarly, offering *ad libitum* feeding to sows one week before parturition maximized feed intake throughout lactation, prevented excessive body condition losses of sows and improved piglet performance (**Chapter 6**, Neil, 1996). Furthermore, body condition of sows (Revell et al., 1998a) and composition of the lactation diet can affect milk composition (Quiniou et al., 2008) affecting milk energy and protein output (Hansen et al., 2012).

8.2 Peripartal scoring system: is there a problem?

Based on the findings of our research and all different parts of the peripartal syndrome discussed, we tried to develop a scoring system (Box 1) that could be used to evaluate the peripartal situation on modern sow herds. Basically, this scoring system summarizes our findings and translates them towards field conditions.

BOX 1: Peripartal scoring system

1.	Mobilization of back fat during the last week of gestation?	0/1
2.	Back fat thickness of sows above 22 mm when entering the farrowing unit?	0/1
3.	Gestation length less than 114 days?	0/1
4.	Birth interval between two piglets more than 30 min?	0/1
5.	Sows more than 2 days no feces during the peripartal period?	0/1
6.	Drop in voluntary feed intake during the first week of lactation?	0/1
7.	Piglets hungry and restless during the first week of lactation?	0/1
8.	On average more than 0.5 stillborn piglet per litter?	0/1
9.	Preweaning mortality higher than 12%?	0/1
10.	Heterogeneous litters at birth, high variation in weight (more than 1 kg)?	0/1

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1. A catabolic state of sows during late gestation should be prevented as much as possible as this can affect piglet birth weight. Furthermore, excessive mobilization can affect milk production and impair sow's performance of the subsequent reproduction cycle.

2. High back fat thickness at late gestation is associated with several metabolic disorders like slow parturition, peripartal hypophagia, PHS, and reduced piglet performance.

3. Early parturition, defined as parturition before 114 days of gestation, results in higher incidence of stillborn piglets, immature and less viable piglets, and often also reduced milk production.

4. Slow parturition was identified as the main cause of stillborn piglets as those die intrapartum from asphyxia. Moreover, birth assistance, as often applied when dealing with slow parturition, increased the risk for MMA and PHS.

5. Next to excessive back fat thickness, peripartal constipation was mentioned frequently as a risk factor for several peripartal problems and should, therefore, be avoided.

6. With the onset of lactation, sows need tremendous amounts of energy and nutrients for proper milk production. A drop in feed intake during this first week of lactation will almost always be detrimental for milk production.

7. Whereas MMA affected sows show clinical symptoms like fever, anorexia, or swollen udders, PHS is often missed because of the lack of symptoms observed with the sows. However, piglets of PHS affected sows are hungry and restless, and often this is the only indication of the occurrence of PHS.

8. As stillborn piglets mostly died during parturition, their occurrence should be limited and when several stillborn piglets within one litter were observed, this could be an indication of disturbed parturition process.

9. Preweaning piglet mortality is an indication of lactational performance of the sow. When mortality exceeds 15 % due to non pathogenic reasons, the cause of death should be evaluated. Based on either starvation or crushing as major cause of death feeding strategies should be adjusted in order to reduce the problem.

10. Several authors identified litter heterogenity as an important factor determining piglets colostrum intake and preweaning performance. Therefore, litters should be homogenized as much as possible.

Although validation of this scoring system is highly recommended, a scoring of more than 5/10 could be interpreted as problematic.

8.3 Future perspectives

With this research project it was tried to gain more insight into the peripartal metabolism of the sow. However, studies were often performed on sow herds that did not report any peripartal problems. Therefore, it would be interesting to validate some of our hypotheses under field conditions and preferably on herds suffering from a particular problem.

For example:

- What is the effectiveness of fish oil supplementation on herds dealing with preterm parturition?

  On a sow herd with more than 10 % of sows farrowing before day 114 of gestation a parturition diet containing 2 % of fish oil could be tested in comparison to the regular applied feeding strategy.

- Which risk factors are associated with early parturition?

  By means of a questionnaire on several sow herds with more than 10 % of sows farrowing before day 114 of gestation a risk analysis could be performed to identify potential risk factors for early parturition.

- What is the importance of progesterone persistence post partum in the occurrence of PHS?

  Comparison of progesterone levels of sows suffering from PHS with progesterone levels of normal lactating sows (same breed, parity, body condition and litter size) could clarify the importance of progesterone in the occurrence of PHS.

- Which metabolic parameters could predict whether sows will be susceptible to PHS or not?

- …

One of the most striking conclusions is that *ad libitum* feeding of peripartal sows could be a suitable solution for the catabolic energy status of highly prolific sows at late gestation. However, scientific knowledge considering this feeding strategy is barely published and certainly deserves more attention. Therefore, it would be worthwhile to test this feeding strategy on several sow breeds to confirm the positive production results. Additionally, it would be interesting to investigate the effect of *ad libitum* feeding on both colostrum and milk quality of sows.

Finally, it would be interesting to further test the peripartal scoring system under field conditions and develop it towards a usefull tool for farmers to evaluate their

peripartal sow performance. By developing an online tool farmers could easily fill out a lot of information could be gathered and the scoring system could be improved. Possibly, a weighting should be added to the scoring system give a more reliable and correct score. More research considering the appropriate solutions for these problems is warranted, as at present knowledge is often limited to identification of risk factors.

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Summary

Summary

As a result of intensive genetic selection for increased numbers of total born piglets, the modern highly prolific sow has to face a huge metabolic challenge. Besides proper housing and adequate health care, adequate feeding of these sows is of utmost importance to prevent metabolic disorders and production losses. Although a lot of research on the effect of nutrition on the sow's metabolism was performed over the past decades, most studies focused either on gestation or lactation diets. However, on the specific feeding strategies during the transition period from gestation to lactation, also defined as the peripartal period, literature is limited (**Chapter 1**). Appropriate peripartal feeding has the potential to improve sow productivity and there are indications that some specific feeding strategies (both related to feed composition and feeding scheme) had the potential to improve metabolism. Therefore, the general aim of this PhD research was to investigate how targeted peripartal feeding strategies could contribute to the prevention of frequently occurring peripartal problems (**Chapter 2**). Also the metabolism behind nutritional manipulation was studied to obtain a better understanding of the pathophysiology of these peripartal problems.

It was described in literature that by altering the fatty acid profile of the diet the metabolism around parturition could be affected. By supplementation of fish oil shortly before parturition, gestation length and piglet viability could be improved. Results of these previously published fish oil trials, however, were equivocal and also clarity about the optimal dosage was needed. Therefore, in **Chapter 3**, different dosages of pork lard, ranging from 0 to 4 %, were exchanged for fish oil in a basal parturition diet. Feeding different dosages of fish oil over a period of 9 consecutive days resulted in a dose-responsive incorporation of the fed n-3 polyunsaturated fatty acids (PUFA) in the membranes of erythrocytes. However, no effects on reproductive performance were detected except for a decrease of piglet performance (increased mortality and reduced weaning weight). Furthermore, with increasing amounts of fish oil erythrocytes were more susceptible to oxidative damage and also oxidative parameters measured in the plasma of sows (thiobarbituric acid reactive substances and ferric reducing ability of plasma) increased. Whether this increased oxidative susceptibility forms the basis for the reduced piglet performance is not clear. These results, however,

indicated the importance of adequate provision of anti-oxidants together with fish oil and a dosage of 2 % of fish oil was considered as the optimal dosage for peripartal diets.

A second frequently occurring probem in sows is hypophagia shortly after parturition. Although some risk factors for this problem were identified, the physiological background was poorly understood. Given that feed intake regulating hormones such as leptin, ghrelin, and resistin, were not only involved in feeding motivation of sows, but they were also related to reproduction via their role in the energy metabolism. When monitoring the peripartal profile of leptin, ghrelin, and resistin in relation to back fat thickness of peripartal sows on the one hand, and to the feeding scheme (restricted versus *ad libitum*) on the other hand, a clear time dependent peripartal profile of all three hormones was observed (**Chapter 4**). Except for elevated leptin levels of sows with more than 22 mm of back fat at parturition, no correlation between the three measured hormones and the actual feed intake or the sow's body condition was recorded. Also no actual hypophagia was observed, not even when one or two risk factors (high feed intake and/or fat body condition) were present. Therefore, further research on the physiological background of peripartal hypophagia should be performed, preferably in sows suffering from hypophagia.

Peripartal sows are often in negative energy balance, not only as a result of problematic feed intake during early lactation, but also because of increased energy demands for fetal growth at the end of gestation. To optimize the nutrient intake of sows, the digestibility of the diet could be improved by adding N,N-dimethylglycine (DMG, **Chapter 5**). This choline-derived metabolite potentially affecting glucose metabolism was added to a parturition diet. Although DMG failed to affect the glucose metabolism of the post-partum sow, it improved apparent fecal digestiblity when supplemented at 1000 ppm/kg feed. Despite the improvement of nutrient digestibility, no effects of reproductive performance were detected in this study.

Besides improved digestibility, increased feed intake during the peripartal peirod could be another strategy to deal with the lack of energy intake of peripartal sows. When feeding sows *ad libitum* starting 5 days pre-partum, sows were capable of regulating their own feed intake (**Chapter 6**). These *ad libitum* fed sows consumed twice as much feed compared to sows fed according to a commonly restricted feeding scheme. However, on the day of farrowing they

reduced their voluntary feed intake from 9 to 4 kg. Monitoring the metabolism of these sows showed that regardless of feeding scheme, several metabolites related to fat, protein, and bone metabolism rapidly changed throughout the peripartal period. Moreover, except for elevated levels of triglycerides for fat sows (more than 22 mm back fat) the metabolite profiles were not affected by body condition of the sows. Although no big differences in metabolism were observed, sows fed *ad libitum* were more capable of maintaining their body condition throughout lactation. Hence, their piglets were also heavier at weaning except when sows had high levels of back fat at parturition. These fat sows mobilized more back fat than their leaner counterparts and consumed equal amounts of feed, but their piglets were lighter at weaning. Based on the results of this study, *ad libitum* feeding of peripartal sows could be a valuable method to prevent sows becoming catabolic around parturition and to improve productivity.

One of the most underestimated peripartal problems was probably constipation. Given that this painful condition was identified as a potential risk factor for several other peripartal problems, impaired hindgut transit should be avoided. It is well established that fibres in gestation diets improved gut health and increased lactational feed intake. However, it was not yet investigated which type of fibre is most suitable for the prevention of peripartal constipation. Therefore, the effect of different types of fibres (sugar beet pulp, wheat bran, and raw potato starch) included in peripartal diets was investigated (**Chapter 7**). In accordance with feeding sugar beet pulp throughout gestation, lactational feed intake was improved by feeding this fibre during the peripartal period. Furthermore, this study pointed out that raw potato starch resulted in the lowest incidence of constipated sows. Additionally, this fibre fermented in the hindgut, resulting in the production of short chain fatty acids which were used as alternative energy source by the peripartal sow. As a result of using raw potato starch, sows fed this fibre were more capable of maintaining their back fat thickness in comparison to wheat bran or sugar beet pulp fed sows. But despite these interesting findings, no effects on reproductive performance were detected.

In conclusion, it can be stated that modern highly prolific sows often have to cope with different peripartal problems. Although several individual problems were identified, most of them were related to each other and in practice, they seldom occur independently form each other. Therefore, it was decided to catalogue all these problems as the sow peripartal syndrome (**Chapter 8**).

SUMMARY

Research in this PhD was a first attempt to affect the sow's metabolism using targeted feeding and to prevent them suffering from the sow peripartal syndrome.

Samenvatting

Doorgedreven genetische selectie in de zeugenhouderij heeft ervoor gezorgd dat de totale nestgrootte drastisch is gestegen. Echter, als gevolg hiervan is ook de metabole belasting voor de zeug enorm toegenomen. Ter preventie van metabole stoornissen is het, naast optimale huisvesting en een goede diergeneeskundige begeleiding, van groot belang dat hoog-productieve zeugen optimaal gevoederd worden. De afgelopen jaren is er veel onderzoek verricht naar het effect van zeugenvoeding op de productiviteit, vruchtbaarheid en metabolisme, maar in de meeste studies wordt er gefocust op het drachtvoeder of het lactatievoeder (**Hoofdstuk 1**). Studies die het effect van specifieke voederstrategieën nagaan voor de overgangsperiode tussen dracht en lactatie zijn echter zeer schaars. Uit dit gebrek aan informatie omtrent voedersamenstelling en -schema's voor peripartale zeugen is het idee voor dit doctoraatsonderzoek gegroeid. De vraag was hoe aangepaste voeding (zowel wat samenstelling als schema betreft) kon bijdragen tot het voorkomen van enkele vaak voorkomende peripartale problemen bij zeugen (**Hoofdstuk 2**). Bijkomend werd ook extra aandacht besteed aan hoe voeding het metabolisme van de zeug beïnvloedde zodat een beter inzicht in de fysiologie verkregen werd.

Het gebruik van visolie als bron van n-3 meervoudig onverzadigde vetzuren in zeugenvoeding is duidelijk beschreven in literatuur. Het is tevens beschreven dat supplementatie van visolie bij peripartale zeugen het metabolisme van de zeug kan beïnvloeden, de drachtduur kan verlengen en de vitaliteit van de biggen kan verbeteren. De resultaten in de literatuur zijn echter niet eenduidig. Verder is er ook onduidelijkheid omtrent de optimale dosis en de eventuele randvoorwaarden die noodzakelijk zijn voor een optimale werking van visolie. In **Hoofdstuk 3** werd onderzocht wat het effect is van een werpvoeder met verschillende dosissen visolie, gaande van 0 tot en met 4 %, uitgewisseld tegen varkensvet om voeders isocalorisch te houden. Wanneer deze verschillende voeders gedurende 9 opeenvolgende dagen gevoederd werden aan peripartale zeugen was er een duidelijke dosisrespons incorporatie van de n-3 meervoudig onverzadigde vetzuren in de celmembranen van rode bloedcellen. Maar in tegenstelling tot andere publicaties werd geen verschil in reproductieparameters waargenomen, met uitzondering van een verminderde prestatie bij de biggen (hogere mortaliteit en lager speengewicht). Daarenboven bleek de gevoeligheid

voor oxidatieve schade lineair toe te nemen met stijgende concentratie visolie in het voeder. Of deze gestegen gevoeligheid voor oxidatieve schade aan de basis ligt voor de gedaalde biggenprestaties, is echter niet duidelijk. Maar uit de resultaten van deze proef kan wel geconcludeerd worden dat het belangrijk is om naast verhoging van het aandeel n-3 meervoudig onverzadigde vetzuren de nodige antioxidanten te voorzien. Tot slot kan aangenomen worden dat een dosis van 2 % visolie optimaal bleek voor gebruik in werpvoeders voor zeugen.

Een tweede vaak voorkomend probleem bij zeugen is gedaalde voederopname kort na de partus. Hoewel enkele risicofactoren voor dit probleem geïdentificeerd zijn, is er tot op heden weinig geweten omtrent de fysiologische achtergrond van dit probleem. Een mogelijke verklaring kan gezocht worden in de verandering van enkele voederopname regulerende hormonen namelijk leptine, ghreline en resistine. Deze drie hormonen regelen niet alleen de vrijwillige voederopname maar zijn tevens ook betrokken bij de metabole sturing van de voortplanting via hun aandeel in het energiemetabolisme. Uit de resultaten van **Hoofdstuk 4** blijkt dat zowel de concentratie van leptine, ghreline als resistine duidelijk veranderen tijdens de peripartale periode. Maar, met uitzondering van hogere leptinewaarden voor zeugen met meer dan 22 mm rugspekdikte, konden geen verschillen in deze hormoonprofielen vastgesteld worden in functie van de conditie van de zeugen (bepaald aan de hand van de rugspekdikte) of het voederschema (*ad libitum* versus beperkt gevoederd). Verder werd tijdens deze studie ook bij geen enkele zeug een daling in voederopname waargenomen, zelfs niet wanneer één of meerdere risicofactoren (hoge voedergift kort voor werpen of vette lichaamsconditie) aanwezig waren. Daarom kan uit deze studie geconcludeerd worden dat, alhoewel het profiel van de drie onderzochte hormonen duidelijk veranderde tijdens de peripartale periode, er vermoedelijk andere factoren meespelen in het geval van peripartale hypofagie. Onderzoek bij zeugen die effectief lijden aan peripartale hypofagie zou meer inzicht kunnen brengen, aangezien de aanwezigheid van twee van de risicofactoren niet voldoende bleek te zijn.

Aangezien van problematische voederopname in het begin van de lactatie worden zeugen vaak katabool op het einde van de drachtperiode als gevolg van toenemende nutritionele behoeftes van de foeti. Daarom is het belangrijk om de nutriëntenvoorziening voor de peripartale zeugen zo optimaal mogelijk te houden. Het verhogen van de verteerbaarheid van het voeder voor peripartale

zeugen is één van de mogelijkheden die kan bijdragen tot een verbeterde nutriëntenvoorziening (**Hoofdstuk 5**). Door toevoeging van N,N-dimethylglycine (DMG) aan het werpvoeder kan niet enkel de verteerbaarheid verbeterd worden, maar kan mogelijk ook het glucosemetabolisme beïnvloed worden via methyldonatie. Hoewel DMG supplementatie (1000 ppm/kg) niet leidde tot veranderingen in het glucosemetabolisme van peripartale zeugen, was een duidelijke verbetering van de schijnbaar fecale verteerbaarheid merkbaar. Ondanks deze verbeterde verteerbaarheid kon echter geen verbetering in de reproductieresultaten waargenomen worden.

Naast een verbetering van de verteerbaarheid kan een verhoogde nutriëntenvoorziening ook bekomen worden door het verhogen van de voedergift. In **Hoofdstuk 6** werd een frequent toegepast gerantsoeneerd voederschema voor peripartale zeugen vergeleken met het *ad libitum* voederen vanaf 5 dagen prepartum. Over de totale peripartale periode was de voederopname van *ad libitum* gevoederde zeugen dubbel zo hoog als van beperkt gevoederde dieren. Verder werd ook duidelijk dat wanneer zeugen zelf kunnen bepalen hoeveel ze eten, ze zichzelf beperken op de dag van werpen en nadien hun opname weer opdrijven. Een vergelijking van de metabolieten aangaande vet-, eiwit- en botmetabolisme gemeten bij beide groepen zeugen toonde merkwaardig genoeg weinig verschillen. Wanneer rekening gehouden werd met de conditie van de zeugen, gemeten als rugspekdikte vlak voor het werpen, kon enkel een verhoging in triglyceridewaarden bij vettere zeugen (rugspekdikte > 22 mm) waargenomen worden. Niettegenstaande de afwezigheid van grote verschillen in metabolieten was het duidelijk dat *ad libitum* gevoederde zeugen beter in staat waren om hun conditie te behouden tijdens de lactatie ondanks een gelijke voederopname gedurende de rest van de lactatie. Bovendien waren de biggen van de *ad libitum* gevoederde zeugen op het moment van spenen zwaarder dan deze van de beperkt gevoederde dieren, op voorwaarde dat zeugen niet meer dan 22 mm ruspek hadden op het einde van de dracht. Op basis van de resultaten van deze studie kan geconcludeerd worden dat *ad libitum* voederen van zeugen rond het werpen mogelijk potentieel biedt om te voorkomen dat zeugen katabool worden en als gevolg hiervan minder productief.

Een laatste, vaak onderschat probleem waarmee veel zeugen rond het werpen te kampen hebben, is constipatie. Aangezien deze pijnlijke aandoening genoemd

wordt als potentiële risicofactor voor enkele andere peripartale problemen is het belangrijk om dit probleem zoveel mogelijk te vermijden en te streven naar een optimale darmgezondheid. Het is algemeen aanvaard dat toevoegen van voldoende vezels aan het voeder van drachtige zeugen niet alleen de darmgezondheid maar tevens ook de vrijwillige voederopname tijdens lactatie beïnvloedt. Maar ondanks deze kennis is het niet duidelijk welk soort vezel best gebruikt wordt. Daarom werden in een laatste studie drie verschillende types vezel (suikerbietenpulp, tarwezemelen en rauw aardappelzetmeel) met elkaar vergeleken (**Hoofdstuk 7**). Wanneer gebruik gemaakt werd van een werpvoeder rijk aan suikerbietenpulp werd, in overeenstemming met eerder onderzoek naar suikerbietenpulp in drachtvoeder, een verbetering in vrijwillige voederopname tijdens lactatie waargenomen. Rauw aardappelzetmeel was in staat om de incidentie van peripartale constipatie te verlagen en bijkomend resulteerde de fermentatie van deze grondstof in de vorming van korte keten vluchtige vetzuren in de dikke darm. Deze vluchtige vetzuren konden door de zeugen gebruikt worden als alternatieve energiebron waardoor ze beter in staat waren om doorheen de peripartale periode hun rugspekdikte te behouden.

Tot slot kan geconcludeerd worden dat veel van de problemen waarmee peripartale zeugen te kampen hebben met elkaar verband houden. Zelden wordt in de praktijk slechts een enkel probleem gemeld en kunnen deze verschillende problemen samen benoemd worden als het peripartaal syndroom. Onderzoek in dit doctoraat was een eerste aanzet om via doelgerichte voeding het metabolisme van de zeug te beïnvloeden en te voorkomen dat moderne hoog-productieve zeugen vatbaar zijn voor het peripartaal syndroom.

Curriculum Vitae

Curriculum Vitae

An Cools werd geboren op 28 augustus 1981 te Mechelen. In 1999 behaalde zij haar diploma secundair onderwijs in de richting Wiskunde-Wetenschappen aan de Ursulinen te Mechelen en aansluitend startte zij haar studies Bio-ingenieur aan de KULeuven. In 2005 behaalde zij het diploma van Bio-ingenieur in de Landbouwkunde (major Dierproductie, minor Tropische Landbouw) met onderscheiding.

Gedurende 2 jaar was zij werkzaam als adviseur varkensvoeding en milieuadviseur. In februari 2008 trad zij in dienst als Dehousse bursaal aan de vakgroep Voeding, Genetica en Ethologie en in januari 2009 behaalde zij een IWT specialisatiebeurs. Gedurende ruim 5 jaar deed zij onder meer onderzoek naar het effect van werpvoeders bij zeugen aan deze vakgroep, in samenwerking met de Vakgroep Verloskunde, Voortplanting en Bedrijfsdiergeneeskunde. Daarnaast verstrekte zij frequent nutritioneel advies voor verschillende bedrijven en instellingen, dierenartsen en adviseurs. Ze werkte mee aan de opleiding van studenten.

An Cools is auteur en mede-auteur van meerdere wetenschappelijke publicaties in internationale tijdschriften. Tevens was zij spreker op verschillende nationale studiedagen en internationale congressen.

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Dankwoord

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