Piglet birth weight & litter uniformity

Importance of pre-mating nutritional and metabolic conditions

J.G.M. [Anne] Wientjes

Thesis committee

Promotor

Prof. dr. ir. B. Kemp
Professor of Adaptation Physiology
Wageningen University

Co-promotors

Dr. ir. N.M. Soede
Associate professor, Adaptation Physiology Group
Wageningen University

Dr. ir. H. van den Brand
Associate professor, Adaptation Physiology Group
Wageningen University

Other members

Prof. dr. ir. W.H. Hendriks, Wageningen University
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Prof. dr. G. Janssens, University of Ghent, Belgium
Dr. H. Quesnel, Institut National de la Recherché Agronomique (INRA), Saint-Gilles, France

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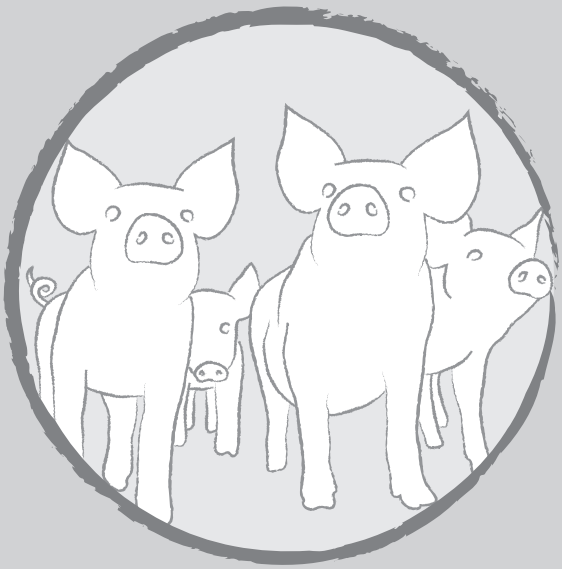
ABSTRACT

High piglet birth weights and litter uniformity are important for piglet survival and piglet performance. Within-litter variation in piglet birth weight is the consequence of within-litter variation in early embryo development, which in turn reflects variation in follicle and oocyte development. Insulin-stimulating diets before mating can influence litter development and uniformity, probably through beneficial effects of insulin on IGF-1 and follicle development. The first aim of this thesis, therefore, was to study effects of insulin-stimulating diets during the weaning-to-estrus interval (WEI) on plasma insulin and IGF-1 levels and follicle development, and consequences for embryo, fetal and placental development and uniformity at different stages of pregnancy in sows. Results of this thesis show that plasma insulin levels during WEI can be effectively enhanced by dietary sugars as dextrose and sucrose (high peaks directly after feeding) and starch (enhanced insulin levels at ~4h after feeding) in a dose-dependent manner. Follicle development and subsequent litter uniformity of embryos (at day 10 of pregnancy) or fetuses and placentas (at day 42 of pregnancy), however, were not affected by insulin-stimulating diets during WEI, nor related to plasma insulin and IGF-1 levels during WEI. Besides effects of pre-mating diets, plasma insulin and IGF-1 levels and follicle development are also influenced by the pre-mating metabolic state of the sow: in sows with severe body condition loss during lactation, plasma insulin and IGF-1 levels and follicle development at weaning are suppressed, and restoration of plasma insulin and IGF-1 levels and follicle development occurs in sows with a prolonged lactation or a prolonged weaning-to-pregnancy interval (WPI). The second aim of this thesis, therefore, was to study effects of these pre-mating conditions related to sow metabolic state on subsequent piglet birth weight and litter uniformity. In this thesis it is shown for the first time that pre-mating conditions related to sow metabolic state affect subsequent litter uniformity. Litter uniformity at birth was compromised by severe sow body condition loss during previous lactation and improved in sows with a prolonged WPI. Furthermore, it was shown that in (organic) sows with prolonged lactations (6 weeks) and large litters (17.4±0.3 piglets), insulin-stimulating diets before mating did not result in improved piglet birth weights or litter uniformity. This may be related to a restored follicle development at weaning in these sows. In these large organic litters, piglet birth weight and litter uniformity were strongly related to piglet survival during lactation. To conclude, results of this thesis confirm that litter uniformity at birth is already (partly) determined during the pre-mating period, likely related to (insufficient) restoration of follicle development. In contrast to previous studies, insulin-stimulating diets during WEI did not improve litter uniformity of embryos, fetuses or placentas and/or piglets in sows. The role of plasma IGF-1 levels and follicle development at weaning (both related to sow parity and sow body condition loss), and effects of insulin-stimulating diets during lactation, need further study. Finally, although effects of pre-mating nutritional and metabolic conditions on subsequent piglet birth weight and litter uniformity seem only marginal, these marginal effects can have substantial effects on pre-weaning piglet survival.

Contents

Chapter 1

General introduction



1.1 PIGLET SURVIVAL

In pig breeding, profitability is largely determined by the number of piglets reared per sow per year. Over the last decades, sows have been strongly selected on litter size, which has resulted in average litter sizes of 13 - 15 total born piglets (Figure 1.1). This increase in litter size, however, is associated with an increase in pre-weaning piglet mortality. In nowadays European pig production, one out of five piglets born dies before weaning. In The Netherlands in 2011, for example, from the on average 14.8 total born piglets 1.1 piglets (~ 7.5%) were born dead, and from the on average 13.7 liveborn piglets 1.8 piglets (~ 13.0%) died before weaning (Figure 1.1). Reduced piglet survival, therefore, is a major problem in commercial pig husbandry, both from an economic and welfare point of view.

1.2 PIGLET BIRTH WEIGHT AND LITTER UNIFORMITY

Important factors for pre-weaning piglet survival are piglet birth weight and litter uniformity (Milligan et al. 2002; Quiniou et al. 2002; Damgaard et al. 2003). Piglets with a low birth weight have a lower chance of survival (Milligan et al. 2002; Quiniou et al. 2002; Damgaard et al. 2003), because they are physiologically compromised in terms of energy reserves and thermoregulatory capacity, have a delayed and lower colostrum intake (and thereby impaired passive immunity) and a disadvantage in competing with heavier littermates at the udder [i.e. a lower ability to get the best teats; Tuchscherer et al. (2000);

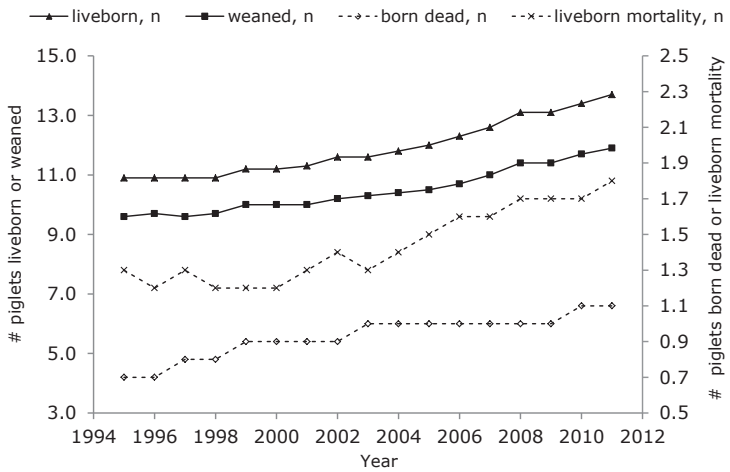


Figure 1.1 Average technical results of Dutch sow farms [based on "Dutch Technical Results"; Agrovision BV (2012)].

Baxter and Edwards (2012); Quesnel et al. (2012)]. Low birth weight piglets, therefore, are more susceptible to chilling, starvation and crushing by the sow compared to heavier piglets. Low litter uniformity, i.e. large within-litter variation in piglet birth weights, is also associated with low piglet survival (Milligan et al. 2002; Quiniou et al. 2002; Damgaard et al. 2003). This is on the one hand related to the fact that litters with a low uniformity contain more low birth weight piglets, which have a low chance of survival, but also because low birth weight piglets may have a competitive disadvantage within less uniform litters. Milligan et al. (2002) have shown that a decreased piglet survival with decreasing litter uniformity in birth weight was especially evident in litters with low mean birth weight.

Piglet birth weight and litter uniformity are not only important factors for piglet survival, but also for piglet performance before and after weaning. Low piglet birth weight and low litter uniformity result in lower piglet growth during lactation and lower and more variable piglet weights at weaning (Milligan et al. 2002; Quiniou et al. 2002; Beaulieu et al. 2010), as well as reduced and more variable growth after weaning (Quiniou et al. 2002; Gondret et al. 2005; Berard et al. 2008; Rehfeldt et al. 2008; Beaulieu et al. 2010). Detrimental effects of low birth weight on meat and carcass quality are also reported (Gondret et al. 2006; Rehfeldt and Kuhn 2006; Rehfeldt et al. 2008).

1.3 FACTORS AFFECTING PIGLET BIRTH WEIGHT AND LITTER UNIFORMITY

Piglet birth weight and litter uniformity are both negatively related to litter size. For each additional piglet in a litter, mean piglet birth weight decreases with approximately 30 - 35 g (Van der Lende and De Jager 1991; Quiniou et al. 2002; Quesnel et al. 2008; Beaulieu et al. 2010). Within-litter variation in birth weights, measured as the variation coefficient of birth weights (CV), increased from approximately 15 - 17% in small litters (< 10 - 12 piglets) to approximately 24% in large litters [> 15 piglets; Quiniou et al. (2002); Quesnel et al. (2008)].

Piglet birth weight and litter uniformity are also affected by parity (Milligan et al. 2002; Damgaard et al. 2003; Quesnel et al. 2008). Mean piglet birth weight is usually higher in litters from 2nd to 4th parity sows compared to younger and older sows, whereas litter uniformity generally decreases with parity (Damgaard et al. 2003; Quesnel et al. 2008). It is not clear, however, to what extent the parity effect on litter uniformity is related to

differences in litter sizes among parities. Although both piglet birth weight and litter uniformity are associated with litter size and parity, Quesnel et al. (2008) concluded that litter size, parity, sow birth year and season at conception together explained only 20% of the variation in litter uniformity at birth, indicating that other factors must be involved.

Breed differences in piglet birth weight and litter uniformity do exist (Finch et al. 2002; Damgaard et al. 2003; Canario et al. 2009). Meishan sows, for example, usually have lower and more uniform piglet birth weights compared to other (commercially used) sow breeds, despite higher litter sizes (Finch et al. 2002; Canario et al. 2009). Canario et al. (2009) reported mean birth weights of 1330 g in Meishan vs. 1520 g in Large White sows, and within-litter birth weight CV of 17.7% in Meishan vs. 20.7% in Large White sows, with corresponding litter sizes of 12.3 in Meishan vs. 11.0 in Large White sows. Mean piglet birth weight (h^2 = 0.30 - 0.50) has a higher heritability than litter uniformity [SD and CV $h^2 < 0.10$; Högberg and Rydhmer (2000); Hermesch et al. (2001); Damgaard et al. (2003); Wolf et al. (2008); Kapell et al. (2011)]. Also at the phenotypic scale, there is considerable repeatability over successive litters in mean piglet birth weight (Quesnel et al. 2008; Knol et al. 2010; Foxcroft 2012), but repeatability of litter uniformity is low (Quesnel et al. 2008).

Recently, Campos et al. (2012) reviewed effects of sow feed intake during pregnancy on piglet birth weight, and found that effects are inconsistent. So far, there are no indications that feed intake during pregnancy can influence litter uniformity (Cassar et al. 1994; Musser et al. 2004; Cerisuelo et al. 2008; Quesnel et al. 2010).

1.4 THE ORIGIN OF WITHIN-LITTER BIRTH WEIGHT VARIATION

Fetal growth, and thereby piglet birth weight, is largely determined by nutrient supply from the placenta, which in turn is largely determined by placental size (Biensen et al. 1999; Town et al. 2005; Foxcroft et al. 2009; Vallet et al. 2009). The implantation site, and thereby placental size, is determined by the available uterine space at implantation (from ~ day 12 of pregnancy), and is largely fixed at ~ day 35 of pregnancy; thereafter pig fetuses can hardly benefit from newly available uterine space (Vonnahme et al. 2002; Vallet et al. 2009, 2011). Placental size, and thereby its uniformity among littermates, at as early as day 35 of pregnancy, therefore, will be reflected in subsequent fetal growth and litter uniformity. Indeed, Van der Lende et al. (1990) made plausible that the within-litter weight distribution at birth is already established at day 35 of pregnancy.

In the pig, uniformity in placental size is directly related to uniformity in embryo development at implantation at ~ day 12 of pregnancy (Geisert and Schmitt 2002; Vallet et al. 2009). That is because the more developed embryos start to elongate earlier and occupy more than an equal share of the uterine space, ultimately resulting in a larger placenta. The slower/less developed embryos may either die (due to asynchrony with the continuously changing uterine environment, triggered by the more developed embryos) or occupy a less than equal share of the uterine space, ultimately resulting in a smaller placenta. Litter uniformity at birth, thus, seems to be largely determined already during the (pre-)implantation period.

A pronounced diversity in morphological stage of development exists already among littermate embryos during the pre-implantation period (Perry and Rowlands 1962; Pope and First 1985). Nissen et al. (2000) and Xie et al. (1990a) showed a pronounced diversity among littermates already at the 1- to 4-cell stage of development (< 24h after ovulation), whereas Soede et al. (1992) showed a pronounced diversity among littermate embryos at 70 -110 h after ovulation. Indications exist that early embryo uniformity reflects follicle and oocyte uniformity (Pope 1988; Pope et al. 1990; Xie et al. 1990a, b). Xie et al. (1990a, c), for example, showed that follicular development (based on steroid content), oocyte maturation and early zygotic development were similarly skewed, with a majority being further developed than a lesser developed minority. Pope (1988) demonstrated that destruction of oocytes of later-ovulating (less developed) follicles eliminates the lesser developed embryos normally present at day 11 of pregnancy. Furthermore, Xie et al. (1990b) indicated that oocytes of later ovulating (less developed) follicles became the lesser developed embryos at day 4 of pregnancy, whereas lesser developed embryos at day 4 of pregnancy subsequently became the lesser developed embryos at day 12 of pregnancy. This indicates that follicle and oocyte development and their uniformity, and thus the pre-mating period, may be important for subsequent development and uniformity of embryos and placentas, which in turn affects development and uniformity of piglets at birth.

1.5 IMPORTANCE OF NUTRITION IN THE PRE-MATING PERIOD

1.5.1 Effects of pre-mating diets

Evidence for the importance of the pre-mating period comes from studies in which nutrition of the sow in the pre-mating period influences subsequent development and uniformity of fetuses and piglets. Ashworth et al. (1999b) showed that gilts fed a high feeding

level (3 x maintenance) during the estrus cycle prior to mating had an improved blastocyst development (increased blastocyst cell number) and uniformity (lower within-litter SD in blastocyst surface area) at day 12 of pregnancy compared to gilts fed at maintenance level. Ferguson et al. (2006) fed gilts high fiber diets (containing 50% sugarbeet pulp) during the estrus cycle prior to mating and found a higher uniformity in fetal weight at day 27 of pregnancy, as indicated by a reduced number of litters containing inadequately grown fetuses (i.e. outliers to the left, which amounted to 50% and 75% of litters in gilts fed the high fiber and control diet, respectively). Van den Brand et al. (2006) supplemented dextrose (150 g/day) to the diet of sows (1st to 5th parity) during the weaning-to-estrus interval and found a reduction in subsequent within-litter birth weight variation (- 3.7% in birth weight CV). In a follow-up experiment, in which dextrose plus lactose (both 150 g/day) were fed during lactation and the weaning-to-estrus interval, comparable positive effects on litter uniformity were found (- 3.2% in birth weight CV) and also mean piglet birth weight was higher in the dextrose plus lactose supplemented sows [+ 89 g; Van den Brand et al. (2009)]. The mechanism behind effects of pre-mating diets on piglet birth weight and litter uniformity is not clear, but a possible mechanism involved is graphically shown in Figure 1.2 and will be described in the following section.

1.5.2 Possible mechanism involved in effects of pre-mating diets

It is hypothesized that the effects of pre-mating diets on subsequent piglet birth weight and litter uniformity (see Paragraph 1.5.1) are related to the stimulating effect of these diets on insulin and Insulin-like Growth Factor-1 (IGF-1). A high feeding level, as used by Ashworth et al. (1999b), is known to enhance plasma insulin (Flowers et al. 1989) and IGF-1 levels (Thissen et al. 1994). Sugarbeet pulp, as used by Ferguson et al. (2006), enhanced plasma insulin levels for a prolonged period after feeding in gilts (Vestergaard 1997). Supplementation of dextrose, as used by Van den Brand et al. (2006, 2009), to the diet of either gilts or lactating sows resulted in faster and higher insulin peaks after feeding (Van den Brand et al. 1998; Zieçik et al. 2002), and also plasma IGF-1 levels were increased in sows fed a dextrose-rich diet during and after lactation (Van den Brand et al. 2001).

Insulin and IGF-1

Insulin is produced by pancreatic β-cells and plays a key role in glucose homeostasis, and thereby stimulates glycogen, lipid and protein synthesis. Insulin is secreted after a meal in response to a rise in blood glucose levels. By inhibiting hepatic glucose production and

stimulating glucose uptake by insulin-sensitive tissues as skeletal muscle and adipose tissue, insulin lowers blood glucose levels and thereby regulates postprandial hyperglycaemia (Van Cauter et al. 1997). Besides feeding level and diet composition, insulin levels, and the insulin pattern during the day, are also directly influenced by feeding frequency.

Most systemic IGF-1 is synthesized by the liver under influence of Growth Hormone (GH), but IGF-1 is also locally produced in a variety of tissues, either or not regulated by GH (Giudice 1992; Hossner et al. 1997). IGF-1, as a growth factor, promotes cellular mitosis and differentiation in a variety of systems and thereby stimulates protein synthesis (Adashi et al. 1985; Clemmons and Underwood 1991; Giudice 1992). The IGF-1 system is comprised of IGF-1, IGF-1 receptors in target cells, and a family of six IGF binding proteins (IGFBP-1, -2, -3, -4, -5, -6). The IGFBPs bind IGF-1, and can thereby either inhibit IGF-1 action (e.g. by sequestering IGF-1) or potentiate IGF-1 action (e.g. by prolongation of IGF-1 half-life and facilitating transport to target tissues). Additionally, IGFBP activity is regulated by IGFBP proteases (Giudice 1992). In contrast to insulin levels, plasma IGF-1 levels are more constant during the day and show a longer latency in response to changes in feeding level or diet composition.

Insulin and IGF-1 are structurally related and overlap in receptor specificity. Thus, insulin can interact with IGF-1 receptors (although with lower affinity than IGF-1) and thereby have growth promoting activities, and similarly, IGF-1 can interact with the insulin receptor and thereby mimic insulin actions (Poretsky and Kalin 1987; Clemmons and Underwood 1991; Giudice 1992; Hossner et al. 1997). Furthermore, plasma insulin and IGF-1 levels are usually positively correlated. During catabolic states, insulin and IGF-1 levels are usually suppressed (Clemmons and Underwood 1991; Thissen et al. 1994). During a period of insulin deficiency, e.g. due to a catabolic state, hepatic GH binding, and thereby GH-stimulated IGF-1 production, is inhibited (Clemmons and Underwood 1991; Thissen et al. 1994). Insulin can improve hepatic GH binding during a catabolic state and thereby stimulate IGF-1 production. In anabolic animals, however, insulin effects on IGF-1 production may only be marginal (Thissen et al. 1994).

It is hypothesized that pre-mating diets affect piglet development and litter uniformity through beneficial effects of insulin and/or IGF-1 on follicle and oocyte development (Figure 1.2). Therefore, first selection, recruitment and ovulation of follicles in pigs, and the hormonal processes involved, will be shortly summarized [as thoroughly reviewed by Kemp et al. (1998)].

Follicle development

The total primordial follicle pool, from which follicles can develop during a sow's lifespan, is already established at 7 days after birth. From this primordial follicle pool, groups of primordial follicles (consisting of an oocyte covered with a flat layer of granulosa cells) start to develop and grow out to antral follicles (with multiple granulosa cell layers and a fluid filled cavity). An activated primordial follicle requires 84 days to reach the antral stage [~ 0.4 mm; Morbeck et al. (1992)]. In an additional 14 days, the newly formed antral follicles grow to a diameter of ~ 3 mm or more (Morbeck et al. 1992). Up to ~ 2 mm in diameter, follicle growth is independent of cyclic gonadotropin variations (Driancourt et al. 1995). Thereafter, follicle growth is regulated by the gonadotropic hormones Follicle Stimulating Hormone (FSH) and Luteinizing Hormone (LH), which are released by the pituitary under influence of Gonadotropin Releasing Hormone (GnRH) from the hypothalamus. Granulosa cells have FSH receptors and FSH induces aromatase activity, which results in transformation of androgens to estrogens; androgens are produced by theca cells surrounding the granulosa cells under influence of LH [as reviewed by Kemp et al. (1998)]. During further development, granulosa cells acquire increasing numbers of LH receptors (Foxcroft and Hunter 1985), and gonadotropin dependency gradually shifts from FSH levels to LH pulses. Results of Driancourt et al. (1995), as an example, indicated that from ~ 2 to ~ 4 mm in diameter porcine follicle growth is largely dependent on FSH, and beyond ~ 4 mm follicle growth is largely dependent on LH pulses and not FSH anymore. During the first weeks of lactation pulsatile LH release in sows is inhibited by the suckling stimuli of the piglets (causing release of endogenous opoids that inhibit GnRH release from the hypothalamus), and therefore antral follicle development is minimal during early lactation [as reviewed by Quesnel and Prunier (1995)]. During ongoing lactation, LH pulsatility gradually restores, which allows follicles to develop up to 4 - 5 mm at weaning (Quesnel and Prunier 1995).

At weaning, the removal of the suckling stimulus, in combination with the stress of separation from the piglets, triggers a shift in the pattern of LH release from the hypothalamus/pituitary system from low frequency high amplitude pulses towards high frequency low amplitude pulses. This shift in LH release pattern triggers the outgrowth of antral follicles (~ 3 mm) to preovulatory size (~ 7 - 8 mm), which takes 4 - 6 days (Morbeck et al. 1992) and is called the follicular phase. This shift in LH release pattern stimulates an abrupt increase in estrogen and inhibin production in follicles that acquired LH receptors on granulosa cells. Inhibin produced by large follicles negatively feedbacks on FSH release

from the pituitary, and as a result, follicles with low numbers of granulosa cell LH receptors will go into atresia due to lack of FSH support. While selected follicles continue to grow to preovulatory size, LH pulse frequency and FSH release gradually decrease. The increased estrogen levels induce a preovulatory LH surge via positive feedback and induce estrus behavior. Most sows show estrus within 4 - 6 days after weaning (Quesnel and Prunier 1995), and ovulation usually occurs at 30 ± 3h after the LH surge (Soede et al. 1994).

Because it takes 84 days for a primordial follicle to reach the antral stage (~ 0.4 mm), and it takes an additional 14 days to grow to a diameter of 3 mm or more (Morbeck et al. 1992), development of follicles that ovulate after weaning starts already during lactation. It is good to realize that the antral follicle pool is heterogeneous in terms of follicle size and follicle fluid steroid content. As a result, follicles destined to ovulate may be at markedly different stages of morphological and biochemical maturation at the time of recruitment, selection and ovulation (Foxcroft and Hunter 1985; Hunter and Wiesak 1990).

Effects of insulin and IGF-1 on gonadotropin release

Positive relations between plasma insulin and LH pulsatility were found in lactating sows (Tokach et al. 1992; Koketsu et al. 1996; Quesnel et al. 1998b; Yang et al. 2000), in cyclic gilts after exogenous insulin administration during the follicular phase (Cox et al. 1987), and in feed restricted prepubertal gilts after realimentation (Booth 1990; Booth et al. 1996). Cox et al. (1987) also observed higher basal LH and FSH levels on the first day of the follicular phase after exogenous insulin administration in cyclic gilts. Positive relations between plasma IGF-1 and LH pulsatility were observed in lactating sows (Van den Brand et al. 2001), and *in vitro* studies support the stimulating effect of IGF-1 on LH release from pig pituitary cells (Whitley et al. 1995; Barb and Hausman 2009). Stimulating effects of both insulin and IGF-1 on FSH and LH release (both basal FSH/LH release and GnRH induced FSH/LH release) are shown in cultured rat pituitary cells (Adashi et al. 1981; Kanematsu et al. 1991; Soldani et al. 1994). This indicates that insulin and IGF-1 may stimulate follicle development via stimulation of gonadotropin release.

Direct effects of insulin and IGF-1 on follicle development

In addition to effects on gonadotropin release, insulin and IGF-1 may also directly regulate follicle development at the ovarian level. Insulin receptors are present at granulosa and theca cells (Otani et al. 1985; Quesnel 1999) and IGF-1 receptors are present at granulosa cells (Maruo et al. 1988; Quesnel 1999).

In *in vivo* studies, positive relations between systemic insulin or IGF-1 levels and follicle development were not necessarily accompanied by increased gonadotropin levels. In lactating first parity sows, with suppressed insulin and IGF-1 levels due to a catabolic state, plasma insulin and IGF-1 levels during late lactation were positively related to ovary weight and mean follicle diameter during the first 3 days after weaning (Quesnel et al. 1998b, 2007; Van den Brand et al. 2001). In cyclic gilts, insulin depletion (due to streptozocin-induced diabetes), which was accompanied by reduced plasma IGF-1 levels, suppressed follicle growth (lower average follicle diameter and lower number of follicles > 3 mm) and steroidogenesis (as indicated by lower follicle fluid estrogen levels), and increased the percentage of atretic follicles (Cox et al. 1994; Edwards et al. 1996). Positive effects of daily exogenous insulin administration during the weaning-to-estrus interval of first parity sows are reported on steroid content in follicular fluid at day 5 after weaning (Whitley et al. 1998). In cyclic gilts, daily exogenous insulin administration during the follicular phase increased ovulation rate (Cox et al. 1987) and prevented atresia, increased the estrogen:progesterone ratio in follicular fluid and increased LH binding of granulosa cells specifically of medium sized (4 - 6 mm) follicles (Matamoros et al. 1990).

In vitro studies support effects of insulin and IGF-1 directly at the ovarian level. In cultured porcine granulosa cells, insulin enhanced glucose metabolism (Otani et al. 1985), and stimulated cell growth and maintenance (May and Schomberg 1981; Baranao and Hammond 1984), cell differentiation alone and in synergy with FSH (May and Schomberg 1981; Amsterdam et al. 1988) and progesterone production alone or in synergy with FSH, LH and estradiol (May and Schomberg 1981; Veldhuis et al. 1983; Baranao and Hammond 1984; Otani et al. 1985; Maruo et al. 1988; Amsterdam et al. 1988; Sekar et al. 2000), and increased the number of LH receptors alone or in synergy with FSH (May and Schomberg 1981; Amsterdam et al. 1988; Maruo et al. 1988; Sekar et al. 2000), whereas in cultured porcine thecal cells insulin stimulated androgen production and progesterone production alone or in synergy with LH (Barbieri et al. 1983). In cultured pig granulosa cells, IGF-1 stimulated cell growth and maintenance (Baranao and Hammond 1984), cell differentiation in synergy with FSH (Maruo et al. 1988), estrogen production in synergy with FSH (Maruo et al. 1988; Howard and Ford 1994) and progesterone production alone and in synergy with FSH, LH and estradiol (Baranao and Hammond 1984; Veldhuis and Demers 1985; Maruo et al. 1988; Sekar et al. 2000), and increased the number of LH receptors alone and in synergy with FSH (Maruo et al. 1988; Sekar et al. 2000). It has to be noted, however, that insulin exerted its effects on cultured pig ovarian cells mostly at supra-physiological levels,

that insulin effects could be mimicked by IGF-1 at lower physiological levels (May and Schomberg 1981; Barbieri et al. 1983; Baranao and Hammond 1984; Maruo et al. 1988), and that effects of insulin and IGF-1 were not additive (Maruo et al. 1988).

Besides effects of systemic IGF-1 levels on follicle development, also an intraovarian autocrine/paracrine IGF-1 system exists. Porcine granulosa cells produce and release IGF-1, which is stimulated by LH, FSH, and other growth factors [e.g. EGF and TGFα; Adashi et al. (1985); Giudice (1992); Spicer and Echternkamp (1995)]. In addition, GH and estrogens can synergize with FSH to stimulate IGF-1 production from porcine granulosa cells (Adashi et al. 1985; Giudice 1992; Spicer and Echternkamp 1995). Follicle fluid IGF-1 levels are reported to increase with follicle size in healthy follicles and are reduced in atretic follicles (Edwards et al. 1996). Further, IGF-1 mRNA levels in porcine granulosa cells are similar for all stages of development in healthy follicles and IGF-1 mRNA is not expressed in atretic follicles (Zhou et al. 1996; Liu et al. 2000). Follicle fluid of porcine follicles also contains IGFBP-2, -3, -4 and -5 (Howard and Ford 1992), which modulate IGF-1 availability and probably inhibit ovarian function by sequestration of IGF-1 (Besnard et al. 1997; Poretsky et al. 1999). Follicle fluid IGFBP-2, -4 and -5 are usually higher in small or atretic follicles compared to large mature follicles (Howard and Ford 1992; Hammond et al. 1993; Grimes et al. 1994; Samaras et al. 1994). Although IGFBP-3 is highly abundant in porcine follicular fluid, IGFBP-3 levels only slightly increase with follicle size and are not related to health status of the follicle (Grimes et al. 1994). Changes in IGFBP levels during follicle growth and atresia are due to changes in ovarian IGFBP production and ovarian IGFBP degradation. The production of IGFBP-2, -4 and -5 by porcine granulosa cells is inhibited by FSH and estrogens, and may be stimulated by insulin and IGF-1 (Giudice 1992; Spicer and Echternkamp 1995). Studies on IGFBP mRNA expression in granulosa cells indicated that IGFBP-2 mRNA is inversely related to follicle diameter (Samaras et al. 1993; Zhou et al. 1996; Liu et al. 2000) and IGFBP-4 mRNA is highly dynamic and closely follows LH receptor mRNA (Zhou et al. 1996; Liu et al. 2000), whereas IGFBP-3 mRNA and IGFBP-5 mRNA were not detected (Zhou et al. 1996). Proteolytic degradation of IGFBP-2, -4, and -5 (not -3) is reported in porcine follicular fluid, which was higher in large preovulatory follicles (6 - 7 mm) compared to small antral follicles (2 mm) and markedly decreased in atretic follicles (Besnard et al. 1997). All together, these intraovarian mechanisms contribute to an increase in intrafollicular IGF-1 availability during development from small antral follicle to preovulatory follicle, whereas intrafollicular IGF-1 availability is markedly reduced in atretic follicles. This strongly supports the concept for an important role of (intraovarian) IGF-1 in follicular development.

There are some indications that systemic insulin and/or IGF-1 levels may affect the intraovarian IGF system. Quesnel et al. (1998a), for example, reported a positive relation between systemic IGF-1 levels and follicular fluid IGF-1 levels. Insulin depletion (due to streptozocin-induced diabetes), which was accompanied by reduced plasma IGF-1 levels, decreased follicular fluid IGF-1 levels in cyclic gilts (Cox et al. 1994; Edwards et al. 1996) and in (PMSG-treated) prepubertal gilts (Meurer et al. 1991). These studies may reflect accumulation of systemic IGF-1 into follicles. Furthermore, exogenous insulin administration increased follicular fluid IGF-1 levels in (PMSG-treated) prepubertal gilts (Matamoros et al. 1991), and Purvis et al. (1997) reported a stimulating effect of insulin on porcine follicular IGF-1 production *in vitro*. This may also indicate that insulin can directly stimulate follicular IGF-1 production.

In summary, there is a growing body of evidence from both *in vitro* and *in vivo* studies that insulin and IGF-1 stimulate follicle growth and steroidogenesis, either indirectly via stimulation of gonadotropin release or directly at the ovarian level, where insulin and IGF-1 can act alone or by amplifying gonadotropin action. Gonadotropins and estrogens, in turn, can also increase ovarian IGF-1 availability, by enhancing ovarian IGF-1 production and inhibiting ovarian IGFBP-2, -4 and -5 activity, and thereby amplify IGF-1 actions in the process of follicle development.

Effects of insulin and IGF-1 on follicle uniformity are not known. Matamoros et al. (1990) showed that during the early and mid-follicular phase, exogenous insulin administration stimulated steroidogenesis and LH binding and prevented atresia of medium sized (4 - 6 mm) follicles, but not of large follicles (> 7 mm). These medium sized follicles still had the potential for either ovulation or atresia. This suggests that especially the lesser developed follicles within the preovulatory follicle pool may benefit from insulin and IGF-1 stimulation, which may result in improved preovulatory follicle uniformity. On the other hand, stimulation of gonadotropins and stimulation of estrogen secretion from the more developed follicles by insulin or IGF-1 may also promote maturation and steroidogenesis of the lesser developed follicles, for example via stimulation of IGF-1 secretion from the more developed follicles (Foxcroft and Hunter 1985; Hunter and Wiesak 1990), and thereby be beneficial for follicle uniformity. Thus, it can be hypothesized that insulin and IGF-1 may not only stimulate follicle development, but may also improve follicle uniformity.

Relation between follicle development and oocyte maturation

Oocytes are arrested in the prophase of the first meiotic division (germinal vesicle stage) until atresia or just before ovulation. At 36 - 40h after the onset of the preovulatory LH surge, oocytes complete the first meiotic division and then enter a second period of arrest (metaphase II) until fertilization (Hunter 2000). Only oocytes in the metaphase II stage can be fertilized. Before leaving the follicle, therefore, nuclear and cytoplasmic maturation of the oocyte must be completed (Hunter 2000). Oocytes and granulosa cells communicate in a bi-directional manner; oocyte maturation is regulated by paracrine signals from granulosa cells and follicle fluid, and pig oocytes regulate granulosa cell function (Hunter 2000; Hunter and Paradis 2009). Hunter and Wiesak (1990) demonstrated that the stage of oocyte maturation was correlated to follicle diameter and estrogen secretion in individual follicles recovered from cyclic gilts during the peri-ovulatory period. Zak et al. (1997a, b) demonstrated that when follicle development was suppressed due to feed restriction during the last week of lactation (as indicated by a suppressed ovulation rate), also a lower proportion of recovered oocytes reached the metaphase II stage after *in vitro* maturation, and recovered follicular fluid was less able to support *in vitro* maturation of oocytes (recovered from prepubertal gilts). The status of the follicle, therefore, may influence oocyte maturation. Stimulation of follicle development and uniformity by insulin and IGF-1, thus, may also improve oocyte maturation and uniformity. This is further supported by the finding that plasma IGF-1 levels during the follicular phase of cyclic gilts were positively correlated to the percentage of oocytes that reached metaphase II after *in vitro* maturation (Ferguson et al. 2003).

Consequences for early embryonic and luteal development

Indications exist that development and uniformity of follicles and oocytes is reflected in subsequent early embryo development and uniformity, as described above (see Paragraph 1.4). More indications come from studies showing that pre-mating nutritional strategies that improve embryo survival, development and uniformity (Zak et al. 1997a; Ashworth et al. 1999b; Ferguson et al. 2006) are also shown to improve follicle development and oocyte maturation (Zak et al. 1997b; Ferguson et al. 2003, 2007).

Besides direct effects of improved (uniformity of) oocyte development, there may also be an indirect effect of improved follicle development on early embryonic development through improved luteal development and progesterone secretion. In the peri-ovulatory period, the follicle starts to produce progesterone, and after the release of the oocyte the

remaining follicle develops into a corpus luteum. Soede et al. (1998) found a positive relation between average follicle volume at ovulation (as measured by ultrasound) and average corpus luteum weight at day 5 of pregnancy in sows, which may indicate that larger and better developed follicles develop into larger and heavier corpora lutea. Heavier corpora lutea may produce more progesterone, as indicated by positive correlations found between total luteal weight and total luteal tissue progesterone content ($r = 0.57$; $P < 0.01$), between total luteal tissue progesterone content and plasma progesterone levels ($r = 0.66$; $P < 0.01$), and between total luteal weight and plasma progesterone levels ($r = 0.50$; $P < 0.01$) at day 5 of pregnancy (NM Soede; unpublished results). Further, pre-mating nutritional strategies that affect plasma insulin and IGF-1 levels, can also influence luteal development (Ashworth et al. 1999a) and progesterone levels (Almeida et al. 2000, 2001; Chen et al. 2012) during early pregnancy in gilts. Progesterone levels during early pregnancy are associated with embryo survival (Ashworth 1991; Foxcroft 1997) and early embryo development (Vallet et al. 1998; Vallet and Christenson 2004) through changes in uterine protein secretion [e.g. Stroband and Van der Lende (1990)]. Thus, improved follicle development may affect progesterone levels, and thereby early embryo survival and development, which in turn may affect placental development and subsequent fetal development and thereby piglet birth weights.

Consequences for placental and fetal development, and finally piglet birth weight and litter uniformity

Development and uniformity in early embryo development is directly reflected in development and uniformity in placental size, which in turn seems to be reflected in fetal development and uniformity, and thereby piglet birth weight and litter uniformity, as previously described (see Paragraph 1.4).

In summary, it is hypothesized that effects of pre-mating diets on piglet birth weight and litter uniformity are related to their insulin and IGF-1 stimulating effects. Pre-mating plasma insulin and IGF-1 levels may improve follicle and oocyte development and their uniformity, which in turn may improve embryo development and uniformity and luteal development, subsequent placental and fetal development and uniformity, and finally piglet birth weight and litter uniformity (Figure 1.2).

1.6 POSSIBLE ROLE OF OTHER PRE-MATING CONDITIONS RELATED TO SOW METABOLIC STATE

Besides effects of pre-mating diets, plasma insulin and IGF-1 levels, and also follicle development and subsequent embryo and fetal development, are influenced by the pre-mating metabolic state of the sow.

During a conventional 3 - 4 week lactation period, sows are in a catabolic state, related to the high nutrient demands for milk production and a limited feed intake capacity (Quesnel and Prunier 1995). This catabolic state during lactation is accompanied by suppressed plasma insulin and IGF-1 levels (Rojkittikhun et al. 1993; Hoving et al. 2012). Severe sow body condition loss during lactation suppresses follicle development at weaning, and – as a consequence – suppresses subsequent reproductive performance [as thoroughly reviewed by Quesnel (2009)]. Negative effects of severe body condition loss during lactation, mainly studied using feed restriction during lactation, are reported on oocyte quality (Zak et al. 1997b), ovulation rate (Zak et al. 1997a; Van den Brand et al. 2000a), embryo survival (Zak et al. 1997a; Van den Brand et al. 2000b; Vinsky et al. 2006; Hoving et al. 2012) and embryo development (Vinsky et al. 2006; Patterson et al. 2011; Hoving et al. 2012). These effects are likely related to compromised follicle development and thereby oocyte quality, because the follicles and oocytes developed during a period of catabolic state [as reviewed by Quesnel (2009)]. Effects on embryo uniformity, or subsequent piglet birth weight and litter uniformity, are not studied before.

After weaning, sows quickly change towards an anabolic state, associated with restoration of plasma insulin and IGF-1 levels (Quesnel et al. 1998a; Van den Brand et al. 2001; Hoving et al. 2012). A prolonged interval from weaning to pregnancy, i.e. a recovery period after weaning, results in higher subsequent litter sizes in sows, as shown for sows with (spontaneous) delayed estrus at > 12 days after weaning (Dewey et al. 1994; Vesseur et al. 1994; Le Cozler et al. 1997; Poleze et al. 2006), for repeat breeders (Tummaruk et al. 2001; Hoving 2012), and also for sows with artificially prolonged weaning-to-estrus intervals through insemination at the 2nd estrus instead of the 1st estrus after weaning (Clowes et al. 1994; Vesseur 1997; Werlang et al. 2011) or through daily administration of a progesterone-analogue (Van Leeuwen et al. 2011), likely because follicles and oocytes developed during a period of anabolic state which benefits their quality. Indeed, an artificially prolonged weaning-to-estrus interval is shown to result in restoration of follicle development (Van Leeuwen et al. 2010, 2011), higher ovulation rates (Patterson et al. 2008), higher

progesterone levels measured 50h after the preovulatory LH surge (Clowes et al. 1994) and improved subsequent embryo and fetal survival and development (Patterson et al. 2008).

Longer lactation lengths, i.e. an increase from 25 days to 40 days, are also associated with higher subsequent litter sizes (Xue et al. 1993; Dewey et al. 1994; Tummaruk et al. 2000). This may be related to a gradual restoration from a catabolic state towards a more anabolic state with prolonged lactation [related to a gradual reduction in suckling frequency over time; Puppe and Tuchscherer (2000)] and thereby restoration of follicle development during lactation. Rojkittikhun et al. (1993), for example, showed that during a 5-week lactation sow body weight loss was higher during the first 3 weeks than during the last 2 weeks, and some sows even gained weight during the last 2 weeks of lactation. Hultén et al. (2002) showed that plasma free fatty acid levels, as an indicator for body fat mobilisation, gradually decreased during the last 3 weeks of a 5-week lactation period in multiparous sows. In The Netherlands, organic sows have longer lactations (on average 42 days in organic and 25 days in conventional sow farms) and farrow larger litters compared to conventional farms using the same sow breed (Leenhouwers et al. 2011), which likely reflects their improved metabolic state and thereby improved follicle development at weaning compared to conventionally weaned sows.

Thus, in sows with severe body condition loss during lactation plasma insulin and IGF-1 levels, follicle development at weaning and subsequent embryo and fetal development are suppressed, whereas restoration of plasma insulin and IGF-1 levels, follicle development and subsequent embryo and fetal development occurs in sows with a prolonged lactation or a recovery period after weaning (i.e. a prolonged weaning-to-pregnancy interval; Figure 1.2). It is not known, however, whether these pre-mating conditions related to sow metabolic state during lactation or after weaning could also affect subsequent piglet birth weight and litter uniformity.

1.7 AIMS AND OUTLINE OF THIS THESIS

Over the last years, litter size has substantially increased to 13 - 15 total born piglets on average. This increase in litter size, however, is associated with an increase in pre-weaning piglet mortality. In nowadays European pig production, one out of five piglets born dies before weaning, which is a major economic and welfare problem. For piglet survival, as well as for piglet performance before and after weaning, high piglet birth weights and litter

uniformity are crucial. Although consequences of piglet birth weight and litter uniformity on piglet survival and performance have been studied, the (biological) causes of within-litter variation and possible factors affecting piglet birth weight and litter uniformity are poorly described. Both piglet birth weight and litter uniformity are affected by litter size, parity and breed, at least in sow populations with average litter sizes up to 13 - 14 total born piglets, but there may be other factors involved. Through linkage of several mechanisms as described above and shown in Figure 1.2, it is hypothesized that piglet birth weight and litter uniformity are already (partly) determined in the pre-mating period. It is hypothesized that pre-mating conditions, and especially pre-mating plasma insulin and IGF-1 levels, affect follicle and oocyte development and uniformity, and thereby subsequent embryo development and uniformity and luteal development, subsequent placental and fetal development and uniformity and finally piglet birth weight and litter uniformity.

Therefore, aims of this thesis are (i) to study effects of insulin-stimulating diets during the weaning-to-estrus interval on plasma insulin and IGF-1 levels, follicle development and uniformity, and consequences for embryo, fetal and placental development and uniformity and luteal development at different stages of pregnancy in sows; and (ii) to study effects of pre-mating conditions related to sow metabolic state during lactation and after weaning on subsequent piglet birth weight and litter uniformity. An improved understanding of these factors and mechanisms affecting piglet birth weight and litter uniformity may lead to the development of new management strategies (e.g. development of pre-mating feeding regimens), which can assist farmers in improving piglet survival and piglet performance.

A series of physiological experiments is used to study whether nutritionally increased plasma insulin and IGF-1 levels during the weaning-to-estrus interval could improve follicle development and uniformity, and subsequent embryo, fetal and placental development and uniformity in multiparous sows. Multiparous sows are used in these physiological experiments, because these sows have a higher incidence of low litter uniformity than younger sows. In order to find the most suitable insulin-stimulating diet to create large contrasts in plasma insulin and IGF-1 levels among sows, in **Chapter 2** effects of different (combinations of) dietary carbohydrate sources on plasma insulin and IGF-1 levels in multiparous sows are evaluated (Figure 1.2). The most suitable insulin-stimulating diet from Chapter 2 is subsequently used to study effects of nutritionally increased plasma insulin and IGF-1 levels during the weaning-to-estrus interval on plasma luteinizing hormone levels and follicle development in **Chapter 3**, and consequences for luteal development, progesterone levels and embryo development and uniformity at day 10 of pregnancy

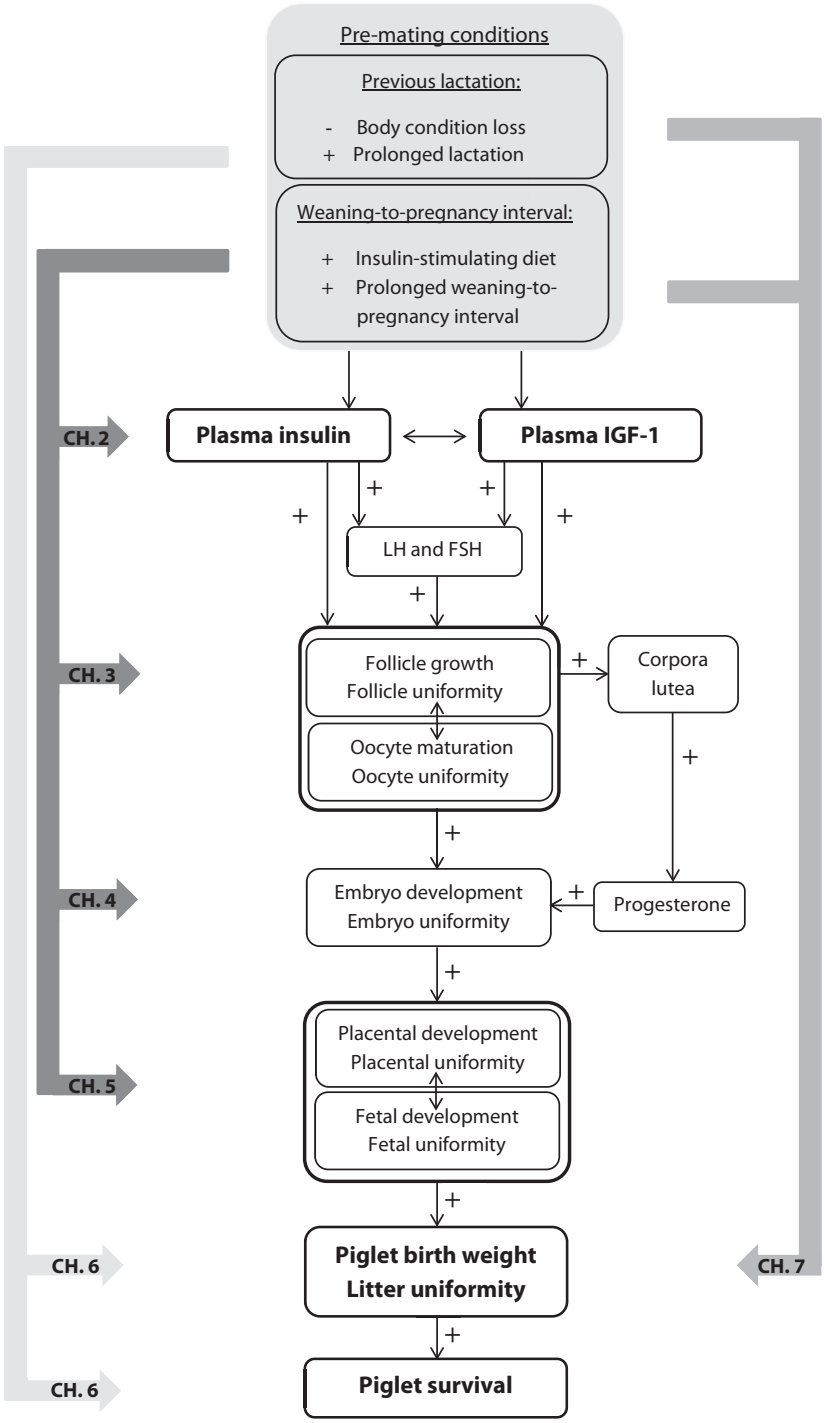


Figure 1.2 Hypothesis and outline of this thesis.

in **Chapter 4** (Figure 1.2). Day 10 of pregnancy (before implantation) is chosen as the first evaluation moment to get a complete overview of the total within-litter variation in embryo development prior to implantation, and to be able to relate uniformity of the preovulatory follicle pool with uniformity of early embryos. After day 10 of pregnancy, part of the within-litter variation present in early pregnancy is lost as a consequence of prenatal losses during the implantation period (~ day 12 - 16). Consequences of different levels of insulin-stimulating feed components during the weaning-to-estrus interval for placental and fetal development and uniformity after the implantation period (at day 42 of pregnancy) are evaluated in **Chapter 5** (Figure 1.2).

To study whether pre-mating conditions related to sow metabolic state during lactation and after weaning could affect subsequent piglet birth weight and litter uniformity, different sow models known to affect plasma insulin and IGF-1 levels and follicle development are used. In **Chapter 6**, piglet birth weights and litter uniformity, and effects of pre-mating insulin-stimulating diets, are studied in organic sows with prolonged lactations (6 weeks; Figure 1.2). These sows likely have an improved metabolic state and improved follicle development at weaning compared to conventionally weaned sows after 3 - 4 week lactation periods. In **Chapter 7**, effects of a prolonged weaning-to-pregnancy interval (used as a model for a recovery period after weaning) and effects of sow body condition loss during lactation in sows with a regular weaning-to-pregnancy interval (≤ 7 days) on subsequent piglet birth weight and litter uniformity are studied in sows with conventional 3 - 4 week lactations (Figure 1.2).

In **Chapter 8**, the findings from Chapters 2 to 7 are combined and discussed to further unravel the biological background of piglet birth weight and litter uniformity, and translated into general conclusions and recommendations.

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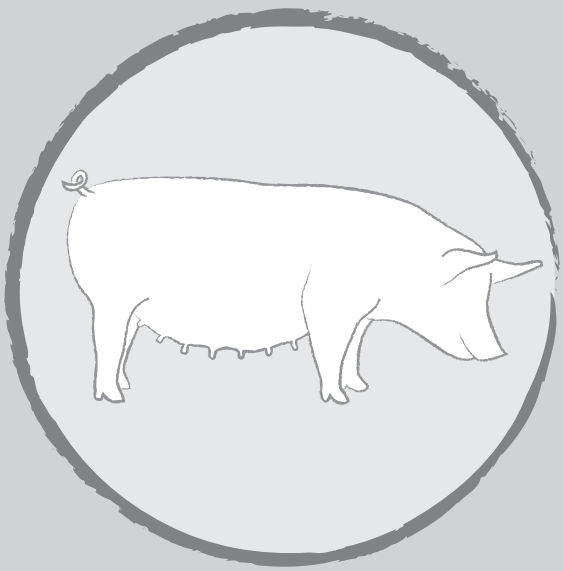
Chapter 2

Effects of dietary carbohydrate sources on plasma glucose, insulin and IGF-1 levels in multiparous sows

JGM Wientjes
NM Soede
F Aarsse
BFA Laurenssen
RE Koopmanschap
H van den Brand
B Kemp

Adaptation Physiology Group,
Department of Animal Sciences,
Wageningen University,
Wageningen, The Netherlands



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ABSTRACT

Effects of different carbohydrate sources on plasma glucose, insulin and insulin-like growth factor-1 (IGF-1) levels were compared to subsequently be able to study effects of insulin-stimulating diets on follicle development in sows. The following feed components were tested in 12 sows during six consecutive test periods of 9.5 days: dextrose (DEX), sucrose (SUC), lactose (LAC), dextrose plus lactose (DL), sucrose plus lactose (SL), dextrose plus sugarbeet pulp (DSBP), and control (CON). On day 2, 5 and 9 of each test period, plasma glucose (only at day 9), insulin and IGF-1 profiles were determined. Despite similar glucose profiles for all diets, the postprandial insulin response was higher for DL and SL than for CON and LAC; the other diets were intermediate. Plasma IGF-1 levels were higher for CON, LAC, and SL than for DSBP, but differences were only marginal. It was concluded that dextrose and sucrose have the potential to stimulate fast and high insulin peaks, especially when combined with additional lactose. Despite the high dextrose in de DSBP diet, the insulin response was flattened, probably due to the viscosity of sugarbeet pulp. The results show that modulation of plasma insulin levels by dietary carbohydrates seems possible in anabolic sows, but IGF-1 levels are less easily modified.

INTRODUCTION

Insulin and insulin-like growth factor-1 (IGF-1) are known to stimulate follicle and oocyte development, either indirectly at the brain level via stimulation of luteinizing hormone (Koketsu et al. 1996; Van den Brand et al. 2001), or directly at the ovarian level (Poretsky and Kalin 1987; Quesnel et al. 2007, 2009).

Insulin and IGF-1 levels can be modulated by diet composition or specific feed components (Vestergaard 1997; Van den Brand et al. 1998, 2001; Zieçik et al. 2002). Various potential insulin-stimulating carbohydrate sources were used to study effects on reproductive performance in sows. A frequently used carbohydrate source is dextrose (Kemp et al. 1993; Van den Brand et al. 2006), either or not in combination with (additional) starch (Van den Brand et al. 2000, 2001; Zieçik et al. 2002). At commercial farms in the Netherlands, sucrose is commonly supplemented to sow diets as an insulin-stimulating feed component, thought to subsequently stimulate reproductive performance. Furthermore, beneficial effects of (partly) fermentable carbohydrate sources such as sugarbeet pulp (Ferguson et al. 2006, 2007) and lactose (Van den Brand et al. 2009) on sow reproductive performance are reported, which might be related to an insulin-stimulating effect of these carbohydrates (Vestergaard 1997). However, in only few of these studies, plasma glucose, insulin and IGF-1 profiles were measured (Kemp et al. 1993; Van den Brand et al. 2000, 2001; Zieçik et al. 2002). For a proper comparison of these different insulin-stimulating carbohydrate sources, and to subsequently find suitable insulin-stimulating diets which can be used in both further research and practice to study or stimulate follicle development, the insulin-stimulating effect of different carbohydrate sources needs to be compared within one study.

Sugars, like dextrose and sucrose, result in a quick and high increase in insulin directly after feeding (Van den Brand et al. 1998; Zieçik et al. 2002), whereas (partly) fermentable carbohydrate sources may increase insulin levels for a prolonged period after feeding (Vestergaard 1997). A diet supplemented with both sugars and fermentable carbohydrate sources seems thus promising for insulin stimulation, as it may enhance both insulin peak levels and peak duration. It is unknown, however, whether effects of sugars and fermentable carbohydrates on insulin profiles are additive or interact with each other.

Therefore, the main objective of this experiment was to evaluate the effects of seven diets, differing in carbohydrate sources, on plasma glucose, insulin and IGF-1 levels in multiparous sows.

MATERIALS AND METHODS

General design

Effects of seven test diets, differing in carbohydrate sources, on glucose, insulin and IGF-1 were tested in 12 multiparous sows during six consecutive test periods of 9.5 days. Within each test period, all seven test diets were fed. On day 2, 5 and 9 of each test period, insulin profiles were determined; glucose profiles were determined at day 9; IGF-1 levels were measured at day 0 (before start of the diet), 2, 5 and 9. Between test periods, sows were fed a basal diet for 4.5 days. All experimental procedures were approved by the Institutional Animal Use and Care Committee of Wageningen University (Wageningen, The Netherlands).

Animals and housing

Twelve multiparous (parity 7.7 ± 0.5; range: 4 - 10) Topigs 20 (Topigs, Vught, The Netherlands) sows arrived at the experimental farm of Wageningen University at 25.8 ± 1.4 days (range: 19 - 33 days) after weaning. After an adaptation period of seven days, the sows were surgically fitted with a permanent jugular vein catheter under general anaesthesia as described by Soede et al. (1997). To prevent estrus and corresponding feed refusals, all sows were given an oral dosage of 40 mg of Altrenogest per day (at the afternoon meal; Regumate, Janssen Animal Health, Beerse, Belgium).

Sows were housed in individual farrowing crates, exposed to 16h of light (0700 - 2300h) and barn temperature was maintained between 18 and 22 °C during the whole experiment. Sows were weighed and P2 backfat was measured at arrival, before catheterization and at day 7 of each test period.

Diets

Seven test diets, differing in carbohydrate sources, were tested during six consecutive test periods of 9.5 days. Dextrose and sucrose were used as sugar sources. Sugarbeet pulp and lactose were used as (partly) fermentable carbohydrate sources. Adult sows have a limited lactase activity in the small intestine (Kim et al. 1978c), and therefore significant amounts of the ingested lactose will flow into the large intestine (Kim et al. 1978c), where it can be (partly) fermented into volatile fatty acids (VFA) or lactate by the microflora (Kim et al. 1978b; Engstrom et al. 1979; Giusi-Perier et al. 1989; Pierce et al. 2006).

Composition of the test diets (manufactured by Research Diet Services BV, Wijk bij Duurstede, The Netherlands) is given in Table 2.1. All test diets were fed at 3 kg/day. Dextrose, sucrose and/or lactose were added to a basal diet (BAS) with sufficient protein, vitamins and minerals (Table 2.1) at a level of 50 g/kg (54 g/kg for dextrose to correct for H_2O adherence), that is 150 g/day (162 g/day for dextrose). The level of 150 g/day was based on studies of Van den Brand et al. (2006, 2009). In those studies, supplementation of 150 g/day dextrose, either or not in combination with 150 g/day lactose, during the pre-mating period had positive effects on subsequent piglet uniformity, possibly related to beneficial effects of insulin on follicle development. To test the effect of each individual carbohydrate source on insulin secretion, the following three test diets were created: DEX (54 g/kg dextrose), SUC (50 g/kg sucrose) and LAC (50 g/kg lactose). To test whether addition of the (partly) fermentable carbohydrate source lactose to a diet containing 150 g/day dextrose or sucrose has an additional effect on insulin secretion, the following two diets were created: DL (54 g/kg dextrose plus 50 g/kg lactose) and SL (50 g/kg sucrose plus 50 g/kg lactose). The control diet consisted of the same BAS diet, containing additional soybean oil (CON; 35 g/kg soybean oil). The dextrose plus sugarbeet pulp diet (DSBP) was a more extreme diet and had a completely distinct composition than the other diets. Vestergaard (1997) showed that a diet containing large amounts of sugarbeet pulp (500 g/kg) resulted in prolonged higher insulin levels from 3 - 4h after feeding onwards compared to a standard diet. Therefore the DSBP diet contained large amounts of sugarbeet pulp (400 g/kg), at the expense of other components of the BAS diet, and also contained a large amount of dextrose (80 g/kg) to result in a large contrast in insulin response compared to the CON diet.

During the experiment, sows were fed two equal portions per day at 0800h and 1530h, and water was available *ad libitum*. From arrival until start of the first test period (9 days after catheterization), sows were fed the BAS diet (Table 2.1) at maintenance level [0.44 MJ ME $kg^{0.75}$/day; Noblet et al. (1990)]. Within each test period, all seven test diets were fed. Sows received a different test diet each test period (six diets tested per sow). To check whether sows needed an adaptation period (which was expected for the (partly) fermentable carbohydrate sources), plasma insulin and IGF-1 levels were measured at day 2, 5 and 9 of each test period. To prevent possible carry-over effects, the BAS diet was fed at maintenance level for 4.5 days between test periods, and for each sow, diets containing lactose (LAC, DL, SL) and diets without lactose (CON, DEX, SUC, DSBP) were fed alternately. On blood sampling days (day 2, 5 and 9), feed refusals were removed at 36 min after feeding. On

Table 2.1 Composition of the experimental diets

	Diet[1]							
Ingredient, g	BAS	CON	DEX	SUC	LAC	DL	SL	DSBP
Wheat	160.1	154.3	148.6	149.2	149.2	143.5	144.1	142.5
Barley	200.0	192.8	185.6	186.4	186.4	179.2	180.0	150.0
Palm kernel expeller (CF > 220 g/kg)	100.0	96.4	92.8	93.2	93.2	89.6	90.0	0.0
Sugarbeet pulp (sugar < 100 g/kg)	125.0	120.5	116.0	116.5	116.5	112.0	112.5	**400.0**
Wheat middlings	160.0	154.2	148.5	149.1	149.1	143.4	144.0	35.0
Soybean meal, extracted (CF < 50 g/kg)	128.0	123.4	118.8	119.3	119.3	114.7	115.2	135.0
Soybean hulls (CF 320 - 360 g/kg)	35.0	33.7	32.5	32.6	32.6	31.4	31.5	0.0
Sugarcane molasses (sugar > 475 g/kg)	50.0	48.2	46.4	46.6	46.6	44.8	45.0	40.0
Vitamin-mineral premix	5.0	4.8	4.6	4.7	4.7	4.5	4.5	5.0
Limestone	7.5	7.2	7.0	7.0	7.0	6.7	6.8	2.5
Monocalciumphosphate	6.0	5.8	5.6	5.6	5.6	5.4	5.4	7.0
Salt	3.0	2.9	2.8	2.8	2.8	2.7	2.7	2.5
L-threonine	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.5
Soybean oil	**20.0**	**55.3**	**36.6**	**36.6**	**36.6**	**17.9**	**18.0**	**0.0**
Dextrose	-	-	**54.0**	-	-	**54.0**	-	**80.0**
Sucrose	-	-	-	**50.0**	-	-	**50.0**	-
Lactose	-	-	-	-	**50.0**	**50.0**	**50.0**	-
Total, g	**1000**	**1000**	**1000**	**1000**	**1000**	**1000**	**1000**	**1000**
Calculated content	g/1000 g	g/1000 g	g/1000 g	g/1000 g	g/1000 g	g/1000 g	g/1000 g	g/1000 g
Dry matter	875.5	879.8	880.1	883.9	883.9	884.1	888.0	881.5
Crude fat	43.8	78.0	58.6	58.7	58.7	39.2	39.4	11.9
Crude protein	152.9	147.4	141.9	142.5	142.5	137.0	137.6	136.5
Starch	238.0	229.4	220.9	221.8	221.8	213.3	214.2	174.7
Sugars	64.9	62.6	109.9	113.1	113.1	160.5	163.7	143.1
kJ NE (for swine)[2]	8550	9460	9180	9220	9220	8940	8980	8970

[1] BAS, basal diet; CON, control diet (BAS + 35 g/kg soybean oil); DEX, dextrose diet (BAS + 54 g/kg dextrose); SUC, sucrose diet (BAS + 50 g/kg sucrose); LAC, lactose diet (BAS + 50 g/kg lactose); DL, dextrose plus lactose diet (BAS + 54 g/kg dextrose + 50 g/kg lactose); SL, sucrose plus lactose diet (BAS + 50 g/kg sucrose + 50 g/kg lactose); DSBP, dextrose plus sugarbeet pulp diet (distinct diet containing 80 g/kg dextrose and 400 g/kg sugarbeet pulp).

[2] According to the Centraal Veevoederbureau (CVB 2003).

remaining days, feed refusals were removed within 1h after feeding and recorded as none (< 10%), moderate (10 - 50%), or high (> 50%) refusals.

Blood sampling

A blood sample was taken at 24 min before the 0800h feeding at day 0 (before the start of each test diet). At day 2, 5 and 9 of each period, blood samples were taken at - 24, - 12, 0, 12, 24, 36, 48, 60, 84, 120, 156, 228, 300, and 372 min relative to the 0800h feeding for determination of glucose (only determined at day 9) and insulin profiles. Because preliminary analyses showed no differences in insulin profiles among sampling days, glucose levels were only analyzed in day 9 samples. Blood samples taken at 24 min before feeding at day 0, 2, 5 and 9 were analyzed for IGF-1.

Blood samples were collected in polypropylene tubes containing 50 µl EDTA solution (144 mg/mL saline; Tritiplex III, Merck Nederland B.V., Amsterdam, The Netherlands), immediately placed on ice after collection and centrifuged at 1710 x g for 10 min at 4 °C. Plasma was stored at - 20 °C until analyses.

Plasma analyses

Glucose and insulin

For glucose analyses, 500 µL 0.3 M trichloroacetic acid was added to 50 µL of plasma for precipitation of protein. After centrifugation at 16000 g for 1 min, glucose levels in the supernatant were analyzed in duplicate with an enzymatic colorimetric assay using the glucose-oxidase-peroxidase method (GOD-PAP) using a commercial kit (Roche Diagnostics Nederland BV, Almere, The Netherlands). Plasma insulin levels were analyzed in duplicate with a commercial RIA-kit (PI - 12K Porcine Insulin RIA-kit, Millipore, St. Charles, USA). The sensitivity was 2 µU/ml, and intra- and interassay CV were 4.6% (n = 84) and 8.5% (n = 21) respectively.

For each sampling day, basal glucose and insulin levels were calculated as the mean value of the three samples taken before feeding (- 24, - 12 and 0 min); maximum glucose and insulin levels were defined as the maximum value during the first 156 min after feeding; the increase in glucose and insulin after feeding was calculated as the difference between maximum and basal levels; mean glucose and insulin levels were calculated as the average glucose and insulin levels of all plasma samples after feeding (from 0 until 372 min)

corrected for the time intervals between samples; the glucose and insulin area under the curve (AUC/6.2h) was calculated as the area above basal glucose and insulin levels, from feeding (t = 0) until 372 min (6.2h) after feeding; and the minimum glucose level was calculated as the minimum value during the first 120 min after feeding.

Insulin-like growth factor-1

Insulin-like growth factor-1 levels were quantified in duplicate, using a commercial kit (IRMA IGF-1 A15729, Immunotech, Marseille, France), after extraction of the samples with ethanol/HCl [as validated by Louveau and Bonneau (1996)]. The sensitivity, intra- and interassay CV were 2 ng/ml, 2.2% (n = 26) and 3.5% (n = 12) respectively.

Statistical analyses

Sows with a (temporary) lack of catheter patency (28 insulin profiles of the potential 216 insulin profiles; i.e. 12 sows x 6 test periods x 3 sampling days) or feed refusals (26 insulin profiles) at the blood sampling days were excluded from analyses. An overview of the numbers of observations (sows and profiles/samples) used for each diet are given in Table 2.2 for glucose and insulin analyses, and in Table 2.3 for IGF-1 analyses.

Data were analyzed with the MIXED procedure of SAS 9.1 (SAS Inst. Inc., Cary, NC). For all MIXED-analyses, first interaction terms were tested and removed from the model when not significant ($P > 0.05$), followed by a stepwise removal of the variable with the highest non-significant P-value ($P > 0.05$), except for the factor diet. Results are presented as LSmeans ± SE, unless otherwise stated.

Glucose and insulin

Plasma glucose and insulin levels were analyzed using model 1: $Y_{ijklm} = \mu + Diet_i + Period_j + Sampling\ day_k + Sampling\ time_l + Diet_i * Sampling\ day_k + Diet_i * Sampling\ time_l + e_{ijklm}$, where μ = overall mean; $Diet_i$ = test diet (i = CON, DEX, SUC, LAC, DL, SL, DSBP); $Period_j$ = test period (j = 1, 2, 3, 4, 5, 6); $Sampling\ day_k$ (only for insulin levels) = day of sampling (k = 2, 5, 9); $Sampling\ time_l$ = time of sampling relative to feeding (l = - 24, - 12, 0, 12, 24, 36, 48, 60, 84, 120, 156, 228, 300, 372 min); $Diet_i * Sampling\ day_k$ (only for insulin levels) = interaction between diet and sampling day; $Diet_i * Sampling\ time_l$ = interaction between diet and sampling time; and e_{ijklm} = residual error. Because multiple observations per sow cannot be regarded as independent units of observations, sow was added as a random

effect. For insulin levels, a significant interaction between diet and sampling time ($P < 0.01$) existed, indicating that the effect of diet differed between the sampling times. For insulin levels, the variance of the error term was not constant over time (variance decreases with time after feeding), therefore statistical differences between diets were tested for each sampling time separately. Glucose parameters (basal, maximum, increase after feeding, mean, AUC/6.2h and minimum) and insulin parameters (basal, maximum, increase after feeding, mean and AUC/6.2h) were also analyzed using model 1, excluding sampling time and its interaction with diet from the model. The ratio between maximum insulin and maximum glucose (I_{max}/G_{max}) and the ratio between insulin AUC/6.2h and glucose AUC/6.2h (I_{AUC}/G_{AUC}) were calculated for day 9, and analyzed with model 1, excluding sampling day, sampling time, and its interactions with diet from the model.

Insulin-like growth factor-1

Insulin-like growth factor-1 levels at day 0 were analyzed with model 1, excluding sampling day, sampling time, and its interactions with diet from the model, to check whether differences existed in plasma IGF-1 levels before the start of the test diets. Insulin-like growth factor-1 levels at day 2, 5 and 9 were analyzed using model 1, excluding sampling time, and its interaction with diet from the model.

RESULTS

General

Body weight and backfat thickness were 264 ± 5 kg (range: 235 - 294 kg) and 15.9 ± 0.6 mm (range: 13.5 - 18.5 mm) before catheterization, respectively, and 281 ± 5 kg (range: 243 - 311 kg) and 21.0 ± 0.9 mm (range: 16.0 - 26.0 mm) at the end of the experiment (88 days later), respectively. All sows included in the analyses had a 100% feed intake at the blood sampling days and no feed refusals (< 10%) on the remaining days in the test periods.

Glucose and insulin

Pre- and postprandial plasma glucose levels (presented in Figure 2.1; for clarity, a and b both contain the CON profile and other profiles are split up between a and b) and glucose parameters (Table 2.2) were comparable for all diets.

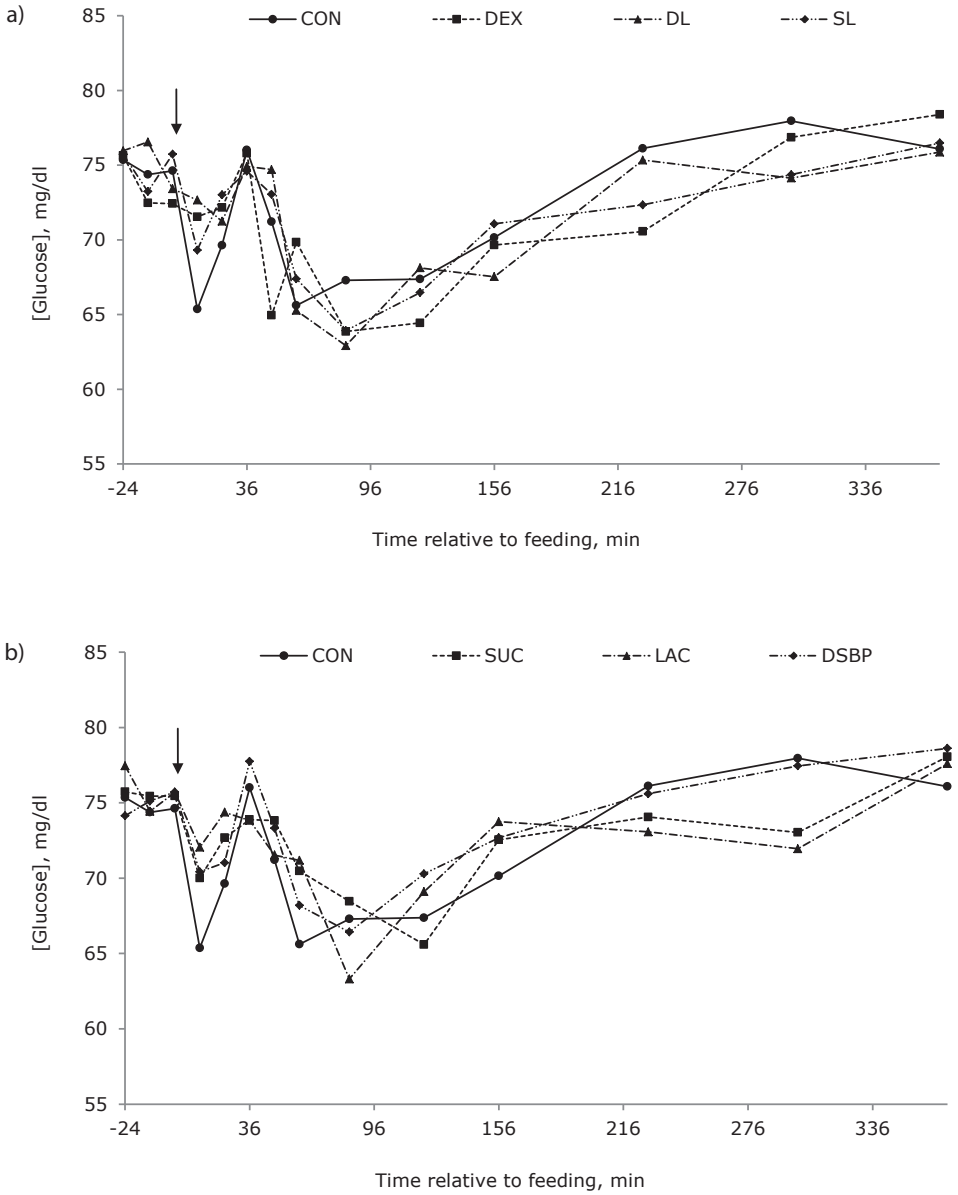

Figure 2.1 Glucose profiles around 0800h feeding for the different diets (LSmeans; corrected for the effects of period and sow), where CON, control diet (55 g/kg soybean oil); DEX, dextrose (54 g/kg) diet; SUC, sucrose (50 g/kg) diet; LAC, lactose (50 g/kg) diet; DL, dextrose (54 g/kg) plus lactose (50 g/kg) diet; SL, sucrose (50 g/kg) plus lactose (50 g/kg) diet; DSBP, dextrose (80 g/kg) plus sugarbeet pulp (400 g/kg) diet; figures are split up in **a)** and **b)** only for clarity, and to compare the test diets with the CON diet, the CON diet is given twice; the arrow indicates the moment of feeding ($t = 0$).

Table 2.2 Effect of the diets on glucose and insulin parameters (LSmeans ± SE)

	Diet[1]							*P*-value[2]		
	CON	DEX	SUC	LAC	DL	SL	DSBP	Diet	Period[3]	Day[4]
Glucose day 9										
Number of sows	9	7	6	8	8	8	7			
Basal, mg/dl	74.4 ± 1.2	73.7 ± 1.4	76.6 ± 1.5	76.6 ± 1.4	75.1 ± 1.3	75.2 ± 1.3	75.1 ± 1.4	0.61	0.01	n.a.
Maximum, mg/dl	78.1 ± 1.8	78.6 ± 2.0	80.4 ± 2.2	78.1 ± 1.9	81.6 ± 1.9	81.5 ± 1.9	83.6 ± 2.0	0.28	-	n.a.
Increase after feeding, mg/dl	3.5 ± 1.6	6.0 ± 1.8	4.1 ± 1.9	3.4 ± 1.7	6.1 ± 1.7	7.4 ± 1.7	7.9 ± 1.8	0.27	-	n.a.
Mean, mg/dl	73.2 ± 1.4	71.1 ± 1.5	72.0 ± 1.6	71.5 ± 1.4	71.0 ± 1.4	71.1 ± 1.4	73.1 ± 1.5	0.64	-	n.a.
AUC, mg/6.2h	- 594 ± 472	- 940 ± 534	- 1504 ± 579	- 1810 ± 523	- 1382 ± 511	- 1421 ± 505	- 517 ± 532	0.31	< 0.01	n.a.
Minimum, mg/dl	59.3 ± 2.4	57.8 ± 2.7	58.3 ± 2.9	59.1 ± 2.5	55.9 ± 2.5	57.8 ± 2.5	58.6 ± 2.7	0.96	-	n.a.
Insulin										
Number of sows	9	7	8	10	9	8	7			
Number of profiles	27	19	20	28	25	23	20			
Basal, µU/ml	8.7 ± 0.6	8.8 ± 0.5	9.8 ± 0.5	8.0 ± 0.5	9.4 ± 0.5	9.6 ± 0.5	8.9 ± 0.5	0.04	0.01	-
Maximum, µU/ml	38.0 ± 4.5^{a}	47.8 ± 5.0^{ab}	43.5 ± 4.9^{ab}	42.7 ± 4.4^{ab}	56.9 ± 4.6^{b}	56.1 ± 4.7^{b}	45.4 ± 4.9^{ab}	< 0.001	-	-
Increase after feeding, µU/ml	29.2 ± 4.5^{a}	39.0 ± 5.0^{ab}	33.7 ± 4.9^{ab}	34.4 ± 4.4^{ab}	47.7 ± 4.6^{b}	46.2 ± 4.7^{b}	36.6 ± 4.9^{ab}	< 0.01	-	-
Mean, µU/ml	16.4 ± 0.9^{ab}	19.0 ± 1.0^{abc}	17.9 ± 1.0^{abc}	15.9 ± 0.9^{a}	19.6 ± 0.9^{c}	19.1 ± 0.9^{bc}	18.4 ± 1.0^{abc}	< 0.01	< 0.01	-
AUC, µU/6.2h	2863 ± 297	3881 ± 348	3018 ± 343	2990 ± 299	3828 ± 312	3517 ± 322	3478 ± 342	0.06	0.01	-
Ratios										
I_{max}/G_{max}[5], µU/mg	48.9 ± 7.8	60.3 ± 8.6	52.9 ± 9.2	55.2 ± 8.1	66.5 ± 8.1	70.1 ± 8.1	68.2 ± 8.6	0.26	-	n.a.
I_{AUC}/G_{AUC}[5], µU/mg	- 3.02 ± 2.58	- 0.99 ± 2.76	- 3.95 ± 2.98	- 1.15 ± 2.58	- 1.91 ± 2.58	- 6.01 ± 2.58	2.53 ± 2.76	0.45	-	n.a.

[1] CON, control diet (55 g/kg soybean oil); DEX, dextrose (54 g/kg) diet; SUC, sucrose (50 g/kg) diet; LAC, lactose (50 g/kg) diet; DL, dextrose (54 g/kg) plus lactose (50 g/kg) diet; SL, sucrose (50 g/kg) plus lactose (50 g/kg) diet; DSBP, dextrose (80 g/kg) plus sugarbeet pulp (400 g/kg) diet.

[2] Statistical significance; - when not significant ($P > 0.05$), factors were removed from the model (except diet); the diet*day interaction was never significant ($P > 0.05$); n.a. not applicable.

[3] Period 1, 2, 3, 4, 5 or 6.

[4] Day 2, 5 or 9 (sampling days for determination of insulin profiles; glucose profiles were only determined at day 9).

[5] I_{max}, maximum insulin; G_{max}, maximum glucose; I_{AUC}, insulin AUC/6.2h; G_{AUC}, glucose AUC/6.2h.

[abc] Within a row, values lacking a common superscript differ ($P < 0.05$).

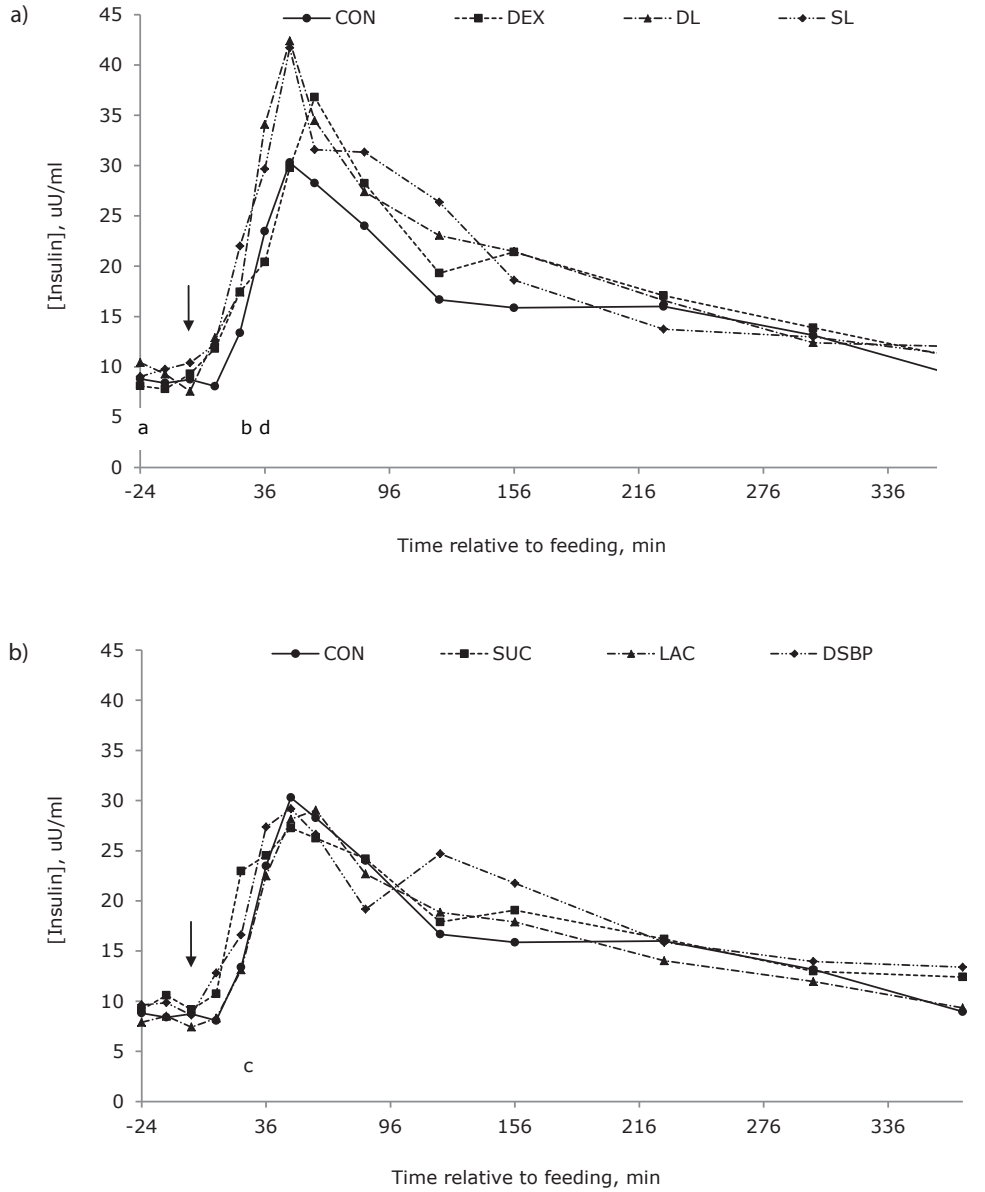

Figure 2.2 Insulin profiles around 0800h feeding for the different diets (LSmeans; corrected for the effects of period, day and sow), where CON, control diet (55 g/kg soybean oil); DEX, dextrose (54 g/kg) diet; SUC, sucrose (50 g/kg) diet; LAC, lactose (50 g/kg) diet; DL, dextrose (54 g/kg) plus lactose (50 g/kg) diet; SL, sucrose (50 g/kg) plus lactose (50 g/kg) diet; DSBP, dextrose (80 g/kg) plus sugarbeet pulp (400 g/kg) diet; figures are split up in **a)** and **b)** only for clarity, and to compare the test diets with the CON diet, the CON diet is given twice; the arrow indicates the moment of feeding (t = 0); significant differences are indicated as follows: a = DL vs. LAC; b = SL vs. CON and SL vs. LAC; c = SUC vs. CON and SUC vs. LAC; d = DL vs. SUC ($P < 0.05$).

Plasma insulin levels and insulin parameters did not differ between the sampling days (day 2, 5 or 9), and therefore average insulin profiles and insulin parameters are presented (Figure 2.2 and Table 2.2 respectively). The effect of diet on plasma insulin levels differed between the sampling times, as indicated by a significant interaction between diet and sampling time ($P < 0.01$; Figure 2.2). Plasma insulin at 24 min before feeding was lower for LAC than for DL (7.8 ± 0.7 vs. 10.8 ± 0.7 μU/ml; $P = 0.02$; Figure 2.2). Postprandial insulin levels were higher for SL and SUC than for CON and LAC at 24 min after feeding (23.7 ± 2.4, 23.0 ± 2.5, 13.4 ± 2.3, and 14.5 ± 2.3 μU/ml for SL, SUC, CON and LAC, respectively; $P < 0.05$; Figure 2.2). At 36 min postprandial, insulin levels were higher for DL than for SUC (34.6 ± 3.6 vs. 21.0 ± 3.9 μU/ml; $P = 0.03$; Figure 2.2).

Basal insulin levels were comparable for all diets (Table 2.2). The postprandial insulin increase (both maximum insulin level and increase after feeding) was higher for DL and SL than for CON (Table 2.2) and mean postprandial insulin levels were higher for DL and SL than for CON and LAC (Table 2.2). Only numerical differences existed in insulin AUC among diets (Table 2.2).

The ratio between maximum insulin and maximum glucose (I_{max}/G_{max}) was comparable for all diets, as was the ratio between insulin AUC/6.2h and glucose AUC/6.2h (I_{AUC}/G_{AUC}; Table 2.2).

2

Insulin-like growth factor-1

Plasma IGF-1 levels at day 0 (before start diet) were comparable for all diets (Table 2.3). Plasma IGF-1 levels were higher at day 2 (156.0 ± 11.8 ng/ml) compared to day 5 (149.9 ± 11.8 ng/ml) and day 9 (149.8 ± 11.8 ng/ml), and IGF-1 levels differed among diets, but the interaction between diet and sampling day was not significant ($P > 0.05$; Table 2.3). Day-corrected IGF-1 levels were higher for CON, LAC and SL than for DSBP, whereas day-corrected IGF-1 levels for DEX, SUC and DL were intermediate (Table 2.3).

DISCUSSION

This study was designed to compare effects of different carbohydrate sources on plasma glucose, insulin and IGF-1 secretion in sows and to subsequently find suitable insulin-stimulating diets that can be used to study effects on follicle development (further research) and stimulate follicle development in practice. Insulin profiles were comparable at day 2,

Table 2.3 Effect of the diets on plasma IGF-1 characteristics (LSmeans ± SE)

	Diet[1]							*P*-value[2]		
	CON	DEX	SUC	LAC	DL	SL	DSBP	Diet	Period[3]	Day[4]
Before start diet (day 0)										
Number of sows	9	9	7	10	9	6	8			
IGF-1, ng/ml	157.4 ± 12.8	157.9 ± 12.7	144.9 ± 13.1	154.6 ± 12.6	154.8 ± 12.8	161.8 ± 13.3	150.8 ± 13.0	0.57	-	n.a.
During the diet (day 2, 5, 9)										
Number of sows	9	9	8	10	10	9	8			
Number of samples	27	23	21	30	28	26	21			
IGF-1, ng/ml	155.6 ± 11.9^{b}	152.9 ± 12.0^{ab}	149.7 ± 12.0^{ab}	157.1 ± 11.9^{b}	150.5 ± 11.9^{ab}	155.5 ± 12.0^{b}	142.1 ± 12.0^{a}	0.01	< 0.01	0.02[5]

[1] CON, control diet (55 g/kg soybean oil); DEX, dextrose (54 g/kg) diet; SUC, sucrose (50 g/kg) diet; LAC, lactose (50 g/kg) diet; DL, dextrose (54 g/kg) plus lactose (50 g/kg) diet; SL, sucrose (50 g/kg) plus lactose (50 g/kg) diet; DSBP, dextrose (80 g/kg) plus sugarbeet pulp (400 g/kg) diet.

[2] Statistical significance; - when not significant ($P > 0.05$), factors were removed from the model (except diet); the diet*day interaction was never significant ($P > 0.05$); n.a. not applicable.

[3] Period 1, 2, 3, 4, 5 or 6.

[4] Day 2, 5 or 9 (sampling days for determination of IGF-1).

[5] At day 2, 5 and 9, absolute IGF-1 levels were 156.0 ± 11.8^{a}, 149.9 ± 11.8^{b} and 149.8 ± 11.8^{b} ng/ml respectively.

[ab] Within a row, values lacking a common superscript differ ($P < 0.05$).

5 and 9 for all diets, which suggests that sows adapted to all diets within 2 days. All sows included in the analyses had a 100% feed intake and feed allowance was similar for all diets. Therefore, differences in insulin and IGF-1 responses between diets cannot be related to differences in feed intake.

Because of our interest in the insulin-stimulating effect of the specific feed components, dextrose, lactose and sucrose were added at the same amount to all diets (150 g/day per carbohydrate source; 162 g/day for dextrose to correct for H_2O adherence), and the daily feed allowance was kept constant for all diets (3 kg/day). Consequently, diets were not completely isocaloric (largest contrast in net energy content 6%), and small differences existed in starch (largest contrast 2%) and fat (largest contrast 4%) content between diets. Additionally, the DSBP diet was a more extreme diet, with a completely distinct composition, designed to create a more extreme contrast in insulin response. However, this resulted in larger differences in starch and fat content between the DSBP and the CON diet (starch: 6%; fat: 7%), making it more difficult to clarify the influence of the individual components on the insulin response of the DSBP diet compared to the CON diet.

Starch is known to have insulin-stimulating effects (Van den Brand et al. 1998; Zieçik et al. 2002). However, Van den Brand et al. (1998) and Zięcik et al. (2002) reported only a numerically higher postprandial insulin response in gilts with a dietary starch contrast of 15 - 20% (starch was exchanged by animal fat). Therefore, in the current study, effects of the – much smaller – differences in starch content among diets on insulin secretion were expected to be negligible. In most studies on effects of starch on insulin secretion, starch was exchanged by fat. However, differences in fat content among diets may modulate the insulin response as well, e.g. through stimulating effects on incretin secretion (such as gastric inhibitory polypeptide and glucagon-like peptide 1; GIP and GLP-1, respectively) in response to absorption of fat (e.g. Ponter et al. 1991; Thomsen et al. 1999), higher plasma free fatty acids levels (e.g. Kveragas et al. 1988) and reduced gastric emptying (e.g. Gentilcore et al. 2006). Incretins such as GIP and GLP-1 (e.g. Collier and O'Dea 1983; Ponter et al. 1991) and free fatty acids (e.g. Itoh et al. 2003) are reported to stimulate insulin secretion, whereas a reduced gastric emptying may flatten the insulin response (e.g. Gentilcore et al. 2006). It is hard to predict whether the differences in fat content between the diets in this study affected postprandial insulin responses *in vivo*, and if yes, whether a higher fat content in these diets would have increased or reduced the insulin response. In this study, soybean oil, consisting mainly of poly-unsaturated fatty acids, was used as fat source. Diets enriched with poly- or mono-unsaturated fatty acids are reported to increase the postprandial insulin response

more than diets enriched with saturated fatty acids (Picinato et al. 1998; Haber et al. 2002), e.g. through stronger stimulating effects of unsaturated fatty acids on incretin secretion (Thomsen et al. 1999; Beysen et al. 2002). Therefore, the contrast in insulin response between the test diets and the CON diet would probably have been larger in case a saturated fatty acid source (e.g. animal fat) would have been used in this experiment. To conclude, in case starch and fat content would have been kept constant for all diets, the contrast in insulin response between the test diets and the CON diet would probably even have been larger, as both the starch and fat content were highest in the CON diet in this study.

The comparable plasma glucose levels and glucose parameters for all diets indicate that sows were able to cope with differences in glucose availability among the diets by secretion of sufficient insulin. Postprandial glucose levels immediately dropped below basal levels. This drop in glucose is probably the result of the quick rise in insulin levels immediately after feeding (before glucose is absorbed), because of e.g. anticipatory neuro-physiological reflexes [i.e. cephalic phase response; as reviewed by e.g. Power and Schulkin (2008)] in combination with the release of incretins (such as GLP-1 and GIP) in response to ingested nutrients (Jenkins et al. 1992; Cummings and Overduin 2007). The drop in glucose was followed by a slight hyperglycaemia, and a marked hypoglycaemia thereafter. This pattern was consistent within all sows and for all diets, and in accordance with results of Pere et al. (2000), and indicates that non-pregnant multiparous sows are highly able to anticipate a postprandial rise in blood glucose and have a high insulin sensitivity.

The DEX diet resulted only in numerically higher maximum (47.8 vs. 38.0 μU/ml) and mean insulin levels (19.0 vs. 16.4 μU/ml) compared to the CON diet, and only insulin levels at 24 min postprandial were significantly higher for the SUC diet than for the CON diet (23.0 vs. 13.4 μU/ml). Kemp et al. (1993) showed that a dextrose-rich diet (24% dextrose) resulted in higher postprandial insulin levels compared to an isocaloric fat-rich diet in gilts. This suggests that dextrose and sucrose are indeed insulin-stimulating feed components, but that the absolute amount supplemented in this study (150 g/day, i.e. 5%) was not sufficient to create a significant higher insulin response than the CON diet in anabolic sows. However, a similar low supplementation of 150 g/day dextrose to sow diets during the weaning-to-estrus interval had positive effects on subsequent piglet uniformity (Van den Brand et al. 2006), suggesting a biological action of this amount of dextrose on follicle development. Unfortunately, insulin profiles were not measured in that study, and therefore, it cannot be ruled out that insulin profiles were differently affected by the 150 g/day dextrose in the recently weaned sows.

Sucrose first has to be hydrolyzed into its monosaccharides glucose and fructose before it can be absorbed. Both glucose and fructose are able to stimulate insulin secretion, but inconsistency exists in literature about the insulin-stimulating effects of sucrose (Swan et al. 1966; Bantle et al. 1983; Helland et al. 1986), or fructose (Bantle et al. 1983; White et al. 1984; Kveragas et al. 1988), compared to glucose (or dextrose). Results of the current study suggest that dextrose and sucrose do not differ significantly in their insulin-stimulating effects in non-pregnant multiparous sows.

Van den Brand et al. (1998) and Zięcik et al. (2002) found significantly higher postprandial insulin responses using diets supplemented with comparable amounts of dextrose (60 g/kg, i.e. 6%) as used in the present study, compared to an isocaloric control (fat-rich) diet. However, they also exchanged starch (15 - 20%; maize starch) between both diets. Ponter et al. (1991) observed a higher postprandial insulin response using a diet high in both sucrose (100 g/kg) and starch (581 g/kg) compared to a fat-rich diet (85 g/kg sucrose; 179 g/kg starch) in pigs. Therefore, dietary supplementation of not only sugars, such as dextrose or sucrose, but also additional starch, may be more effective in stimulating insulin secretion than supplementation of sugars alone.

The diets supplemented with (partly) fermentable carbohydrate sources (LAC and DSBP) did not result in the expected prolonged enhanced insulin levels after feeding. Vestergaard (1997) showed that a diet containing similar amounts of sugarbeet pulp (500 g/kg) significantly enhanced insulin levels above basal levels from 3 - 4h after feeding (until 12h after feeding) in sows. It is possible that we missed long-term effects of the fermentable carbohydrates on insulin in our study, because we only measured insulin levels until 6h postprandial. However, De Leeuw et al. (2004) did not observe a prolonged increase in insulin levels (above basal) in a period up to 11h postprandial in sows fed a diet containing 450 g/kg sugarbeet pulp. Therefore, the effects of fermentable carbohydrate sources on (long-term) insulin secretion in sows needs further investigation.

Pigs older than 4 - 6 weeks of age have a limited lactase activity in the small intestine (Ekstrom et al. 1975; Kim et al. 1978a, c; Rérat et al. 1990; Pierce et al. 2006; He et al. 2008). The level of mucosal lactase activity seems to be independent of the amount of lactose (ranging between 100 and 1600 g) ingested (Rérat et al. 1984) and the duration of lactose inclusion in the diet (Ekstrom et al. 1975; Kim et al. 1978a). This suggests that adult sows are not able to increase their mucosal lactase activity after high or prolonged (> 1 week) lactose ingestion. As a result of the limited lactase activity, significant amounts

of the ingested lactose will flow into the large intestine, as shown by Kim et al. (1978a, b, c), where it will be fermented into VFA or lactate by the microflora, as indicated by (i) increased VFA and lactate production of anaerobically incubated colon and cecum contents (Kim et al. 1978b; Engstrom et al. 1979); (ii) increased VFA absorption from the large intestine into the portal vein (Giusi-Perier et al. 1989); and (iii) increased lactobacilli concentrations in the large intestine (Pierce et al. 2006), after feeding lactose in pigs. It is unknown, however, whether and how long pigs need to adapt to dietary lactose in the diet for an efficient microbial fermentation of lactose, as most studies in pigs only measured lactose digestion after several weeks of adaptation to the lactose diet (Ekstrom et al. 1975; Kim et al. 1978b; Engstrom et al. 1979; Giusi-Perier et al. 1989; Pierce et al. 2006). We did not find a prolonged insulin-stimulating effect of the LAC diet, nor a difference in insulin profiles between sampling days 2, 5 and 9 for the lactose diets (LAC, DL, SL). This suggests that no adaptation to the lactose occurred during the 9-day period in the current study, i.e. that sows were adapted within 2 days. Pierce et al. (2006) showed that 28 g/kg lactose in the diet of pigs resulted in a clear increase in lactobacilli concentrations in the large intestine, and the amount of lactobacilli did not increase further at higher doses of lactose in the diet. This suggests that 50 g/kg lactose in the diet used in the current study should have been sufficient to stimulate lactose fermentation in the large intestine. However, the amounts of VFA and lactate produced in this study may be not sufficient to stimulate (prolonged) insulin secretion.

We expected that the diets supplemented with two different carbohydrate sources, that is a sugar plus a fermentable carbohydrate source (DL, SL, DSBP), would have the largest insulin response, i.e. both high insulin peak levels (sugars) and a prolonged peak duration (fermentable carbohydrates).

Dextrose or sucrose alone (DEX and SUC), i.e. the amount of rapidly available glucose and fructose from hydrolysis of 50 g/kg dextrose or sucrose, did not significantly stimulate insulin secretion compared to the CON diet. Lactose alone (LAC), i.e. the amount of rapidly available glucose and galactose (from the limited lactase activity in the small intestine) and VFA or lactate (from microbial fermentation) from 50 g/kg lactose, did not result in increased insulin levels compared to the CON diet either. But the combination of 50 g/kg dextrose or sucrose with 50 g/kg lactose in the DL and SL diet indeed resulted in significantly higher insulin responses compared to the CON diet. However, this may be (partly) related to the double amount of insulin-stimulating carbohydrates in the DL and SL diet (100 g/kg in total) compared to the DEX, SUC and LAC diets (50 g/kg). We

conclude that the combination of dietary sugars as dextrose or sucrose with lactose within one diet may be beneficial for insulin stimulation.

The DEX and DL diets resulted in higher insulin peaks compared to the CON diet. Dextrose (at 80 g/kg) in the DSBP did not result in such a high insulin peak, which suggests that the insulin-stimulating effect of dextrose was modulated by other nutrients in the DSBP diet, most likely by the high sugarbeet pulp content. Sugarbeet pulp is a water-soluble fiber source, which can form a viscous solution in the gastrointestinal tract (Vahouny and Cassidy 1985). The high viscosity probably delayed gastric emptying (Vahouny and Cassidy 1985; Vestergaard 1997; Serena et al. 2009; Jørgensen et al. 2010), and delayed digestion and absorption of other nutrients (such as glucose) in the small intestine, e.g. through a reduced accessibility of enzymes to these nutrients (Jenkins et al. 1978, 2007; Riccardi and Rivellese 1991; Vestergaard 1997), which consequently resulted in a more flattened glycaemic and insulinaemic response (Jenkins et al. 1978; Wood et al. 1994; Vestergaard 1997; Serena et al. 2009), despite the high dextrose content. We conclude that sugars (such as dextrose) and soluble fibre sources (such as sugarbeet pulp) may negatively interact with each other regarding insulin stimulation when supplemented in the same diet, and thus, the combination of both sugars and soluble fibre sources within one diet do not seem beneficial for insulin stimulation.

Although significant, the differences in IGF-1 levels among the diets (largest contrast 15 ng/ml) seem rather small, and it is questionable whether these differences are biologically relevant. In lactating, catabolic sows, Van den Brand et al. (2001) reported differences in plasma IGF-1 of 31 - 47 ng/ml (depending on sampling day) in primiparous sows fed a dextrose-rich diet compared to a fat-rich diet during lactation. Insulin deficiency, e.g. when feed intake is low and sows are in a catabolic state, inhibits growth hormone (GH)-stimulated IGF-1 synthesis and secretion in the liver, probably through a reduction in hepatic GH binding, i.e. GH resistance (Daughaday et al. 1976; Johnson et al. 1989; Thissen et al. 1994; Lucy 2008). Therefore, in catabolic sows, insulin-stimulating diets (carbohydrate-rich or high energy diets) will improve hepatic GH binding, resulting in stimulation of IGF-1 production. In anabolic sows, however, insulin is probably not limiting for GH-dependent IGF-1 production, which could explain the marginal effects of insulin-stimulating diets found in the current study. Our results suggest that modulation of plasma IGF-1 levels via insulin-stimulating diets seems limited in anabolic, non-pregnant sows.

CONCLUSIONS

Sugar sources as dextrose and sucrose have the potential to stimulate fast and high insulin peaks postprandial. For a more effective insulin stimulation and to subsequently study effects on follicle development, it is recommended to include dextrose or sucrose at higher levels than used in the current study (> 50 g/kg) and/or in combination with additional starch. Furthermore, the combination of dextrose and sucrose with lactose within one diet seems beneficial for insulin stimulation, as the DL and SL diet resulted in the highest postprandial insulin response in this study. However, this might be related to the double amount of insulin-stimulating carbohydrates in the DL and SL diets compared to the DEX, SUC and LAC diets in this study (50 g/kg per carbohydrate source).

Despite the high dextrose in the DSBP diet, the insulin response was flattened, probably related to the high viscosity of sugarbeet pulp in the intestinal tract. Modulation of plasma IGF-1 levels by dietary carbohydrates seems limited in anabolic, non-pregnant sows.

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Chapter 3

Nutritionally induced relationships between insulin levels during the weaning-to-ovulation interval and reproductive characteristics in multiparous sows: I. Luteinizing hormone, follicle development, estrus and ovulation

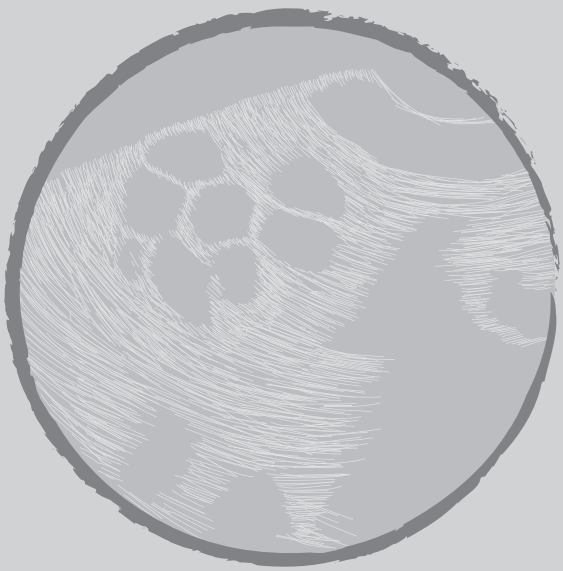
JGM Wientjes

NM Soede

H van den Brand

B Kemp

Adaptation Physiology Group,
Department of Animal Sciences,
Wageningen University,
Wageningen, The Netherlands



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ABSTRACT

To get more insight in how insulin secretion patterns and corresponding insulin-like growth factor-1 (IGF-1) levels are related to luteinizing hormone (LH) secretion, follicle development and ovulation, 32 multiparous sows were fed either a dextrose plus lactose-containing diet at 4h intervals (DL; each 150 g/day) or an isocaloric control diet at 12h intervals (CTRL; containing soybean oil) during the weaning-to-ovulation interval (WOI). Insulin parameters (basal, peak levels, and mean insulin) and IGF-1 levels during the WOI were similar for both treatments, but the insulin secretion pattern differed (related to feeding frequency and meal sizes). Estrus and ovulation characteristics were not influenced by treatment. The LH surge was higher in CTRL than in DL sows (3.73 vs. 3.00 ng/ml; $P = 0.03$). Average diameter (6.5 vs. 6.1 mm; $P = 0.08$) and uniformity (CV: 11 vs. 15%, $P = 0.02$) of follicles ≥ 3 mm at day 4 after weaning was higher in CTRL than in DL sows. Basal insulin levels were positively related to follicle diameter at ovulation ($ß = 0.05$ mm/(μU/ml); $P = 0.04$) and negatively related to LH surge level ($ß = - 0.07$ (ng/ml)/(μU/ml); $P = 0.01$). Insulin area under the curve ($ß = 0.037$ (ng/ml)/1000 μU; $P = 0.02$) and IGF-1 levels ($ß = 0.002$ (ng/ml)/(ng/ml); $P < 0.01$) were positively related to basal LH level around the LH surge. From these data, we conclude that insulin and IGF-1 levels during the WOI are related to LH secretion and follicle development. Not only the absolute level of insulin seems important, but also the pattern within a day in which insulin is secreted seems to affect LH secretion and development of pre-ovulatory follicles.

INTRODUCTION

Recent studies have shown that specific feed components as dextrose and lactose (Van den Brand et al. 2006, 2009) or sugarbeet pulp (Ferguson et al. 2006) in pre-mating sow diets can affect uniformity of fetuses and piglets. Those effects may be mediated through the insulin-stimulating effect of these diets. Insulin and insulin-like growth factor-1 (IGF-1) are known to stimulate follicle and oocyte development, either indirectly at the brain level via stimulation of luteinizing hormone (LH; Koketsu et al. 1996; Van den Brand et al. 2001), or directly at the ovarian level (Poretsky and Kalin 1987; Quesnel et al. 2007).

It is unknown which insulin profiles are optimal for good follicle quality and uniformity, and how these insulin profiles can be achieved. Positive relationships between insulin and LH were found in studies in which sows were fed hourly (Tokach et al. 1992; Koketsu et al. 1996). In studies with twice a day feeding, however, these relationships were less clear (Van den Brand et al. 2000). This indicates that besides the absolute amount of insulin secreted, also the pattern in which insulin is secreted (e.g. prolonged enhanced insulin levels) could play a role in LH stimulation, and thereby follicle development. Besides feeding frequency, insulin secretion pattern can also be modulated by diet composition; e.g. starch plus dextrose-rich diets resulted in a faster, higher and longer insulin peak after feeding compared to fat-rich diets in gilts and sows (Van den Brand et al. 1998, 2001; Zieçik et al. 2002), and sugarbeet pulp enhanced insulin levels for a prolonged period after feeding in gilts (Vestergaard 1997).

To get more insight in how different insulin secretion patterns, and corresponding IGF-1 levels, are related to LH surge characteristics, (uniformity in) follicle development and ovulation, sows were fed either a dextrose plus lactose-containing diet at 4h intervals or an isocaloric and isonitrogenous control diet at 12h intervals during the weaning-to-ovulation interval (WOI).

MATERIALS AND METHODS

General design

During the WOI, multiparous sows were fed either a dextrose plus lactose-containing diet at 4h intervals (DL) or an isocaloric and isonitrogenous control diet at 12h intervals (CTRL; dextrose and lactose were exchanged by soybean oil). In the DL treatment two

factors were combined: specific insulin-stimulating feed components (dextrose plus lactose) and a high feeding frequency (6 x /day). These two factors were used simultaneously to create large contrasts in absolute insulin and IGF-1 levels and their secretion patterns within a day. The focus of this study is on insulin effects on reproductive performance (follicle development, estrus and ovulation), and not on the separate effects of specific feed components or feeding frequency. After ovulation, all sows received a standard pregnancy diet at 12h intervals until slaughter at day 10 after ovulation. All experimental procedures were approved by the Institutional Animal Use and Care Committee of Wageningen University (Wageningen, The Netherlands).

Animals and housing

Multiparous (parity 5.9 ± 0.3; range 3 - 9) Topigs 20 (Topigs, Vught, The Netherlands) sows (n = 38), from one farm, arrived at the experimental farm of Wageningen University within 2h from weaning (day 0), in three consecutive batches. At the day of weaning, sows received either a permanent jugular vein catheter (23 sows) for blood sampling during the whole experimental period, or an ear vein catheter (15 sows) for blood sampling during the WOI. Jugular vein catheters were surgically fitted under general anaesthesia as described by Soede et al. (1997). For insertion of the ear vein catheters, sows were fixated by a nose-sling. A 1.75 m catheter (medical PVC tube, inner diameter 0.8 mm, outer diameter 1.6 mm; Rubber BV, Hilversum, The Netherlands) was inserted 50 cm into the ear vein. The other end of the catheter was passed externally to the back of the sow and a one-way luer-lock stopcock® (Vygon, Veenendaal, The Netherlands) was secured. The ear was fixated at the head of the sow, and sows received 2.2 mg Flunixine® (Intervet Schering-Plough Animal Health, Boxmeer, The Netherlands) kg^{-1} i.m.

Sows were housed in individual farrowing crates during the whole experiment and exposed to 16h of light (0700-2300h).

During the experiment, six sows (four CTRL, two DL) were veterinary treated for pneumonia (five sows) or diarrhea (one sow) and were excluded from all analyses. The remaining sows (n = 32) had an average lactation length of 25.1 ± 0.1 days, a body weight at weaning of 251 ± 5 kg, a lactational body weight loss of 34 ± 1 kg (12.0 ± 0.5%), a backfat thickness at weaning of 15 ± 0.5 mm, a lactational backfat loss of 5 ± 0.3 mm (24.5 ± 1.3%) and weaned 11.6 ± 0.1 piglets.

Dietary treatments

Treatments consisted of two feeding regimes from weaning until 12h after ovulation. These feeding regimens, differing in both specific feed components and feeding frequency, were used to create large contrasts in absolute insulin and IGF-1 levels and patterns among sows. Within two parity classes (≤ 5 or ≥ 6), sows were ranked according to lactational body weight loss (%) and alternately assigned to a dietary treatment: a dextrose plus lactose-containing diet fed at 4h intervals (DL), or a control diet fed at 12h intervals (0800h and 2000h; CTRL).

Either dextrose plus lactose (each 150 g/day), or soybean oil (108 g/day) was added to a basal diet with sufficient protein, vitamins and minerals (Table 3.1). Both diets (Manufactured by Research Diet Services BV, Wijk bij Duurstede, The Netherlands) were fed to be isocaloric

Table 3.1 Composition of the experimental diets (as fed)

Ingredient	DL, g		CTRL, g	
Wheat	159.6		159.6	
Barley	184.1		184.1	
Palm kernel expeller (CF > 220 g/kg)	92.1		92.1	
Sugarbeet pulp (sugar < 100 g/kg)	115.0		115.0	
Wheat middlings	147.3		147.3	
Soybean meal, extracted (CF < 50 g/kg)	105.0		105.0	
Soybean hulls (CF 320-360 g/kg)	33.2		33.2	
Sugarcane molasses (sugar > 475 g/kg)	46.0		46.0	
Vitamin-mineral premix	4.6		4.6	
Limestone	6.9		6.9	
Monocalciumphosphate	5.5		5.5	
Salt	2.8		2.8	
Soybean oil	18.4		45.7	
Dextrose	39.6		-	
Lactose	39.6		-	
Total, g[1]	**1000**		**947**	
Content	Calculated	Analyzed	Calculated	Analyzed
	g/1000 g		g/947 g	
Dry matter	882.1	874.8	832.6	821.6
Crude fat	40.3	39.4	67.4	64.9
Crude protein	136.0	140.4	135.9	137.4
Starch	225.7	222.6	225.6	220.4
Glucose	136.9	113.0	58.8	59.2
kJ NE (for swine)[2]	8860	-	8811	-

[1] 1000 g of the DL diet and 947 g of the CTRL diet are isocaloric and isonitrogenic.
[2] According to the Centraal Veevoederbureau (CVB 2003).

and isonitrogenous. At day of weaning, sows received 1000 g of either the DL-diet or the CTRL-diet (at 1800h). From day 1 after weaning (0800h) the DL-diet was fed in six equal portions of 633 g (3800 g/day). The CTRL-diet was fed in two equal portions of 1800 g (3600 g/day). From 12h after ovulation until slaughter, all sows were fed the basal diet (3000 g/day) at 12h intervals (0800h and 2000h). Water was available *ad libitum* during the whole experiment. One hour after feeding, feed refusals were removed and weighed. Refusal samples were stored at 4 °C and analyzed for dry matter content.

Blood sampling

From day 1 after weaning (0800h) until time of ovulation, blood samples were taken at 4h intervals for all sows to assess the LH surge. IGF-1 levels were determined in 0800h plasma samples at days 1, 2, 3, 4 and 5 after weaning. Furthermore, at days 2 and 3 after weaning, blood samples were taken at -12, 0, 12, 24, 36, 48, 60, 84, 120, 156 and 240 min (for all sows) and at 360, 480 and 720 min (only for CTRL sows) relative to 0800h feeding to assess glucose and insulin profiles.

Blood samples with 100 µl EDTA (Tritiplex III, Merck Nederland B.V., Amsterdam, The Netherlands) solution (0.39M saline) were placed on ice, centrifuged at 1710 x g for 10 min at 4 °C, and plasma was stored at -20 °C.

Follicle development, estrus and ovulation

On day 0 follicle diameter was determined with transrectal ultrasonography (Scanner 200; Pie Medical/Esaote, Maastricht, The Netherlands), by averaging the diameter of the five largest follicles at one ovary. From day 2 after weaning (0800h), estrus detection was performed at 4h intervals by a back-pressure test in the presence of a boar. Time of onset of estrus was defined as 2h before the first time a sow showed a standing response; end of estrus was defined as 2h after the last time the sow showed a standing response. At day 4, ultrasound clips of both complete ovaries were made, using a Mylab 30 scanner (Pie Medical/Esaote, Maastricht, The Netherlands). Diameter and number of antral follicles were analyzed using frame by frame analysis; for each individual follicle the largest diameter of several (≥ 3) consecutive frames was measured. During estrus sows were transrectally scanned at 12h intervals to determine time of ovulation. Time of ovulation was defined as 6h before the first scanning time that no large antral follicles were identified anymore. Follicle diameter at ovulation was defined as the mean diameter of the five largest follicles at the last scanning.

Sows were inseminated every day of estrus with a commercial dose of semen (2 x 10^9 sperm cells), until ovulation had occurred. Sows were slaughtered at the experimental farm 10 days after ovulation. The number of corpora lutea was counted on both ovaries. Data on luteal development and embryos are published elsewhere (Wientjes et al., 2012b).

Plasma analyses

Glucose and insulin

For glucose analyses, 500 µL 0.3M Trichloroacetic Acid (TCA) was added to 50 µL of plasma for precipitation of protein. After centrifugation at 16000 x g for 1 min, glucose levels in the supernatant were analyzed in triplicate with an enzymatic colorimetric assay using the glucose-oxidase-peroxidase (GOD-PAP) method using a commercial kit® (Roche Diagnostics Nederland BV, Almere, The Netherlands). Plasma insulin levels were analyzed in duplicate with a commercial RIA-kit (PI-12K Porcine Insulin RIA-kit®; Millipore, St. Charles, MO, USA). The sensitivity was 2 µU/ml, and intra- and interassay CV were 6.4% (n = 42) and 6.0% (n = 9), respectively.

For each sampling day, basal glucose and basal insulin levels were calculated as the mean value of the two samples taken before feeding (-12 and 0 min); maximal insulin levels were defined as the maximum value during the first 156 min after feeding [when no values were above basal level, no maximum levels were defined (n = 3 sow days)]; the increase in insulin after feeding was calculated as the difference between maximal and basal levels; the area under the curve (AUC) was calculated as the area above basal glucose and insulin levels, where AUC240 was calculated as the area under the curve from feeding until 240 min after feeding for all sows, and for CTRL sows AUC720 was calculated as the area under the curve from feeding until 720 min after feeding; and the mean glucose and insulin level during the sampling period were calculated as the average glucose and insulin levels of all plasma samples after feeding (from 0 until 720 min in CTRL; from 0 until 240 min in DL) corrected for the time intervals between samples.

Insulin-like growth factor-1

Plasma IGF-1 levels were quantified in duplicate, using a commercial kit (IRMA IGF-1 A15729®; Immunotech, Marseille, France), after extraction of the samples with ethanol/HCl [as validated by Louveau and Bonneau (1996)]. The sensitivity, intra- and interassay CV were 2 ng/ml, 2.2% (n = 26) and 3.5% (n = 12), respectively.

Luteinizing hormone

Plasma LH concentrations were analyzed in triplicate, using the homologous double-antibody RIA, as described previously by Cosgrove et al. (1991), with the following modifications: 1% BSA was used in the assay buffer; for the precipitation 50 µL cold Saccel (anti sheep/goat, IDS-AA-SAC2®; Lucron Bioproducts BV, Gennep, The Netherlands) was used; after mixing and incubation for 1h, tubes were centrifuged at 6240 x g for 6 min at 4 °C, aspirated and counted. Porcine LH was obtained from the National Hormone & Peptide Program (NHPP), NIDDK, and dr. Parlow (Harbor-UCLA Medical Center, Torrance, CA, USA). The lower limit of detection was 0.012 ng/ml; the intra- and interassay CV were 6.3% (n = 36) and 4.5% (n = 8), respectively.

Basal LH levels were calculated as the average value of the three lowest values of all samples; LH surge level was defined as the maximum value of all samples.

Statistical analyses

Data are presented as means ± SE. Analysis of variance was applied to continuous data using the GLM procedure of SAS 9.1 (SAS Inst. Inc., Cary, NC, USA). The factors and interactions included in the statistical model were tested for significance and stepwise omitted from the model if $P > 0.10$ (except for the factor treatment), using Bonferroni corrections for multiple comparisons. Pearson correlation was used to analyze relationships among continuous data.

The statistical model for DMI included treatment (CTRL,DL), batch (1,2,3) and the interaction between treatment and batch. Glucose and insulin profiles and parameters were only analyzed for sows with a DMI ≥ 75% (n = 17) at both sampling days. The statistical model included treatment (CTRL,DL), batch (1,2,3), sow nested within treatment and batch, sampling time (-12,0,12,…,240 min), sampling day (2,3), and the 2-way interactions between treatment, sampling time, and sampling day, except that for the parameters sampling time and its interactions were excluded from the model. Because for insulin profiles the variance of error terms was not uniform over time (variance decreases with time after feeding), statistical differences between treatments were tested for each sampling time separately. The statistical model for IGF-1 included treatment (CTRL, DL), batch (1,2,3), DMI_{WOI} (DMI from weaning until 12h after ovulation; dietary treatment period) in classes (where 0 = DMI_{WOI} < 75%, n = 15, and 1 = DMI_{WOI} ≥ 75%, n = 17), sow nested within treatment, batch and DMI_{WOI}, sampling day (1,2,3,4,5), and the 2- and 3-way interactions between treatment, sampling day and DMI_{WOI}.

One sow (DL) developed cystic ovaries (ovulation rate: 3) and was excluded from analyses on follicle development, estrus and ovulation. One sow (DL) had a silent estrus and was excluded from analyses on weaning-to-estrus interval (WEI), estrus duration and follicle diameter at ovulation. The statistical model for follicle development, estrus, ovulation and LH parameters included treatment (CTRL,DL), batch (1,2,3), DMI_{WOI} in classes (where 0 = < 75% and 1 = ≥ 75%), and the interaction between treatment and DMI_{WOI}. Follicle number, diameter and uniformity of the follicle pool (≥ 3 mm) at day 4 after weaning was analyzed on sow level, by merging output of both ovaries to a single observation. Logistic regression was used to analyze follicle diameter as a binary variable (0 = < 5.0 mm and 1 = ≥ 5.0 mm; 0 = < 6.0 mm and 1 = ≥ 6.0 mm; 0 = < 7.0 mm and 1 = ≥ 7.0 mm) using the GLIMMIX macro of SAS, with treatment (CTRL,DL), batch (1,2,3), DMI_{WOI} (0 = < 75%; 1 = ≥ 75%) and the interaction between treatment and DMI_{WOI} as fixed effects and sow added as random effect, using an exchangeable correlation structure.

In all these analyses, relevant covariables and their interaction with treatment (if relevant) were added, e.g. to analyze whether follicle development at day 4 after weaning was related to follicle size at weaning, to analyze relations between insulin and IGF-1 parameters with reproductive parameters, or to analyze effects of body weight losses and backfat losses during lactation on subsequent insulin, IGF-1 and reproductive parameters.

RESULTS

Dry matter intake

DMI_{WOI} did not differ between treatments (63 ± 10% and 73 ± 6% in CTRL and DL sows, respectively; $P = 0.37$). In both treatments, DMI at day 2 0800h and DMI at day 3 0800h were strongly correlated to DMI_{WOI} (overall correlations were $r = 0.88$, $P < 0.001$ and $r = 0.82$, $P < 0.001$ for days 2 and 3, respectively).

Glucose, insulin and IGF-1 profiles during WOI

Glucose levels of sows with a low DMI (< 75%) at both sampling days (days 2 and 3) remained constant after feeding, and no clear postprandial insulin peak could be observed in these sows (data not shown). Glucose and insulin levels and parameters are therefore presented for sows with a DMI ≥ 75% (98 ± 1% in CTRL and 98 ± 2% in DL; n = 17; Figure 3.1 & Table 3.2).

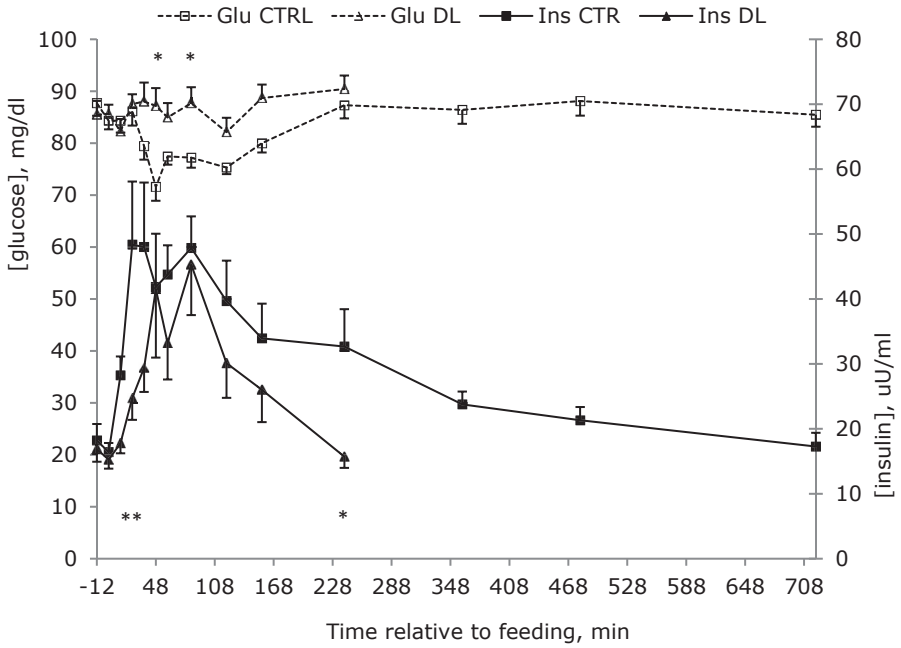

Figure 3.1 Glucose and insulin profiles around 0800h feeding of sows with DMI of ≥75% at days 2 and 3 after weaning, fed either a dextrose and lactose- (each 150 g/day) containing diet at 4h intervals (DL; n = 18 sow days), or an isocaloric control diet at 12h intervals (CTRL; n = 16 sow days) during the WOI (means ± SE); * = CTRL vs. DL, $P < 0.05$.

Glucose

Mean glucose levels were lower in CTRL sows at day 2 than at day 3, and in DL sows at days 2 and 3 (Table 3.2), but the treatment*sampling time*sampling day-interaction was not significant ($P = 0.40$). Figure 3.1 therefore shows average glucose profiles of days 2 and 3 per treatment. Glucose levels were significantly lower in CTRL sows than in DL sows at 48 and 84 min postprandial. Glucose AUC240 was also lower in CTRL sows (- 1699 mg; $P < 0.001$; Table 3.2) than in DL sows.

Insulin

Plasma insulin levels were higher in CTRL sows at 12, 24 and 240 min postprandial than in DL sows, and tended to be higher at 36 min postprandial (Figure 3.1). Insulin AUC240 was also higher in CTRL sows than in DL sows (+ 2165 μU; $P = 0.03$; Table 3.2). Insulin parameters at days 2 and 3 were not related to lactational body weight losses or backfat losses.

Table 3.2 Glucose and insulin parameters of sows with a DMI ≥ 75% at the 0800h feedings of days 2 and 3, for sows fed either a dextrose and lactose- (each 150 g/day) containing diet at 4h intervals (DL) or an isocaloric control diet at 12h intervals (CTRL) during the WOI (means ± SE)

	Treatment		*P*-value[1]	
Item	CTRL	DL	Treatment	Sampling day[2]
Number of sows	8	9		
Glucose				
Basal glucose, mg/dl	86.0 ± 1.6	85.8 ± 1.6	0.25	-
Glucose AUC240, mg	- 1382 ± 365	317 ± 345	< 0.001	-
Glucose AUC720, mg	- 946 ± 1362	n.a.	n.a.	-
Mean glucose[3], mg/dl	84.7 ± 1.6	86.7 ± 1.4	0.29	< 0.01
Insulin				
Basal insulin, µU/ml	17.3 ± 1.2	16.1 ± 1.3	0.59	-
Maximal insulin, µU/ml	74.6 ± 5.9	65.7 ± 7.9	0.48	-
Insulin increase after feeding, µU/ml	57.3 ± 5.8	49.7 ± 7.4	0.51	-
Insulin AUC240, µU	5021 ± 676	2856 ± 374	0.03	-
Insulin AUC720, µU	7596 ± 1190	n.a.	n.a.	-
Mean insulin, µU/ml	27.8 ± 1.6	27.9 ± 2.1	0.98	-

[1] Statistical significance; - when not significant ($P > 0.10$), factors were removed from the model (except treatment); n.a. not applicable.
[2] Day 2 or 3 after weaning (sampling days for determination of glucose and insulin profiles).
[3] Treatment*sampling day interaction ($P < 0.01$); LSmeans CTRL day 2 = 80.1[a] mg/dl, CTRL day 3 = 89.3[b] mg/dl, DL day 2 = 87.6[b] mg/dl, DL day 3 = 86.8[b] mg/dl; ab values with different superscript differ, $P < 0.05$.

3

Insulin-like growth factor-1

Plasma IGF-1 levels did not differ between treatments, but increased from days 1 to 3 after weaning (Figure 3.2). Until day 3 after weaning, IGF-1 levels were independent of DMI_{WOI} (LSmeans were 119.5, 132.9 and 154.5 ng/ml at days 1, 2 and 3, respectively), but IGF-1 levels were higher at days 4 and 5 after weaning in sows with a high DMI_{WOI} than in sows with a low DMI_{WOI} (LSmeans were 174.9 and 152.5 ng/ml at day 4 ($P = 0.01$) and 169.9 and 143.6 ng/ml at day 5 ($P < 0.01$), respectively). IGF-1 levels were positively related to lactational backfat losses at day 2 ($\beta = 6.13$ (ng/ml)/mm backfat loss; $P = 0.06$), day 4 ($\beta = 8.16$ (ng/ml)/mm backfat loss; $P = 0.04$) and day 5 ($\beta = 9.81$ (ng/ml)/mm backfat loss; $P = 0.02$) after weaning.

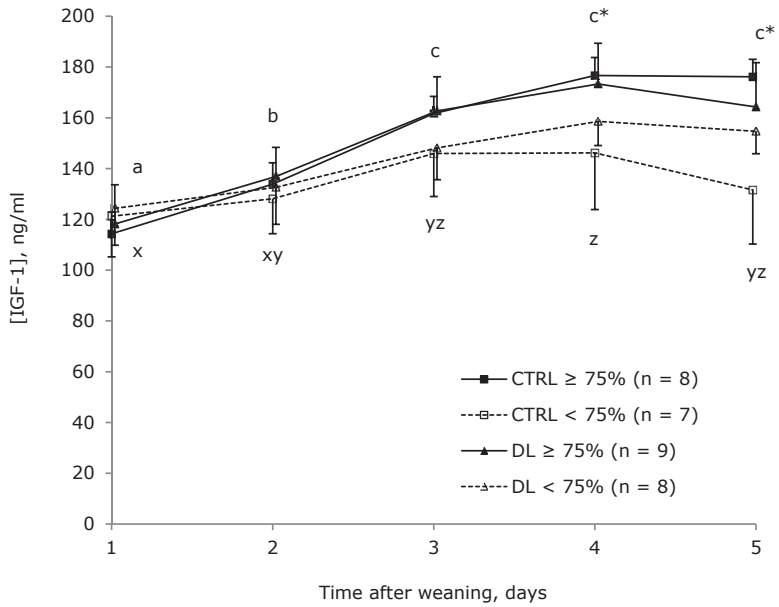

Figure 3.2 IGF-1 profiles from weaning until day 5 after weaning of sows with a high DMI_{WOI} (≥ 75%; as % of total dry matter offered from weaning until 12h after ovulation) or low DMI_{WOI} (< 75%), for sows fed either a dextrose and lactose- (each 150 g/day) containing diet at 4h intervals (DL), or an isocaloric control diet at 12h intervals (CTRL) during the WOI (means ± SE); * effect DMI_{WOI}, $P < 0.05$; abc for sows with high DMI_{WOI} (≥ 75%), days with different superscript differ, $P < 0.05$; xyz for sows with low DMI_{WOI} (< 75%), days with different superscript differ, $P < 0.05$.

Follicle development, estrus and ovulation

Follicle diameter (five largest at one ovary), WEI, estrus duration, WOI, ovulation rate, basal LH and interval weaning-to-LH surge were not influenced by treatment, nor by DMI_{WOI} (Table 3.3). However, LH surge level was higher in CTRL sows than in DL sows (+ 0.73 ng/ml; $P = 0.03$; Table 3.3).

Number of follicles in the follicle pool (≥ 3 mm) at day 4 after weaning was comparable between treatments (21.1 ± 0.8 and 22.1 ± 0.7 in CTRL and DL, respectively; $P = 0.39$), but average diameter of these follicles tended to be higher in CTRL sows than in DL sows (6.5 ± 0.2 and 6.1 ± 0.1 mm, respectively; $P = 0.08$), and uniformity of these follicles was higher in CTRL sows than in DL sows (SD was 0.72 ± 0.04 and 0.88 ± 0.06 mm ($P = 0.05$) and CV was 11 ± 1 and 15 ± 1% ($P = 0.02$) in CTRL and DL sows, respectively). Average diameter of the follicles at day 4 after weaning was not influenced by average follicle diameter at day of weaning and none of the follicle pool characteristics were influenced by DMI_{WOI}.

Table 3.3 Follicle development, estrus and ovulation, and LH characteristics for sows fed either a dextrose and lactose- (each 150 g/day) containing diet at 4h intervals (DL) or an isocaloric control diet at 12h intervals (CTRL) during the WOI (means ± SE)

	Treatment		*P*-value[1]	
Item	CTRL	DL	Treatment	DMI_{WOI}[2]
Number of sows	15[4]	16[4]		
Follicle development				
Follicle diameter at weaning[3], mm	3.3 ± 0.2	3.4 ± 0.1	0.81	n.a.
Follicle diameter at day 4 after weaning[3,4], mm	6.8 ± 0.2	6.8 ± 0.1	0.90	-
Follicle diameter at ovulation[3,4], mm	7.0 ± 0.2	6.9 ± 0.1	0.58	-
Estrus and ovulation				
Weaning-to-estrus interval[4], h	97 ± 3.1	95 ± 3.1	0.67	-
Estrus duration[4], h	57 ± 2.8	54 ± 3.1	0.18	-
Weaning-to-ovulation interval, h	140 ± 3.1	136 ± 3.0	0.40	-
Ovulation rate	24.3 ± 1.2	23.2 ± 0.8	0.43	-
LH				
Basal LH, ng/ml	0.96 ± 0.05	0.92 ± 0.05	0.99	-
LH surge level, ng/ml	3.73 ± 0.24	3.00 ± 0.18	0.03	0.06[5]
Interval weaning to LH surge, h	107 ± 3.1	102 ± 2.5	0.22	-

[1] Statistical significance; the treatment*DMI_{WOI} interactions were not significant; - when not significant ($P > 0.10$), factors were removed from the model (except treatment).
[2] DMI from weaning until 12h after ovulation, as % of total dry matter offered (< 75%, ≥ 75%); n.a. = not applicable.
[3] Average diameter of five largest follicles at one ovary.
[4] Additionally, one DL sow developed cystic ovaries (OR: 3), one DL sow had a silent estrus, and for three sows no ultrasound clips at day 4 were available.
[5] LSmeans were 3.6 and 3.1 ng/ml for sows with a low (< 75%) and high (≥ 75%) DMI_{WOI}, respectively.

3

Distribution of follicles (≥ 3 mm) over six different diameter categories is shown in Figure 3.3. CTRL sows had significantly fewer follicles below 5.0 mm at day 4 after weaning than DL sows (4 ± 2 vs. 13 ± 4%; $P = 0.05$). Also the number of follicles smaller than 6.0 mm was lower in CTRL sows than in DL sows (25 ± 7 vs. 45 ± 6%; $P = 0.04$), but number of follicles larger than 7.0 mm was comparable for both treatments (27 ± 8 vs. 17 ± 5% for CTRL and DL sows, respectively; $P = 0.37$).

Relationships between insulin and IGF-1 with follicle development, estrus and ovulation

In the analyses, the interaction between treatment and insulin parameters (mean values of days 2 and 3) or IGF-1 level (mean value of days 3 - 5 after weaning) was for none of the

reproductive parameters significant ($P > 0.10$), indicating that relationships were comparable for both treatments. Therefore overall treatment corrected regressions ($P < 0.05$) are presented.

Figure 3.4 shows the positive relation between basal insulin and follicle diameter at ovulation ($ß = 0.05$ mm/(µU/ml); $P = 0.04$; Figure 3.4a), and the negative relation between basal insulin and LH surge level ($ß = -0.07$ (ng/ml)/(µU/ml); $P = 0.01$; Figure 3.4b).

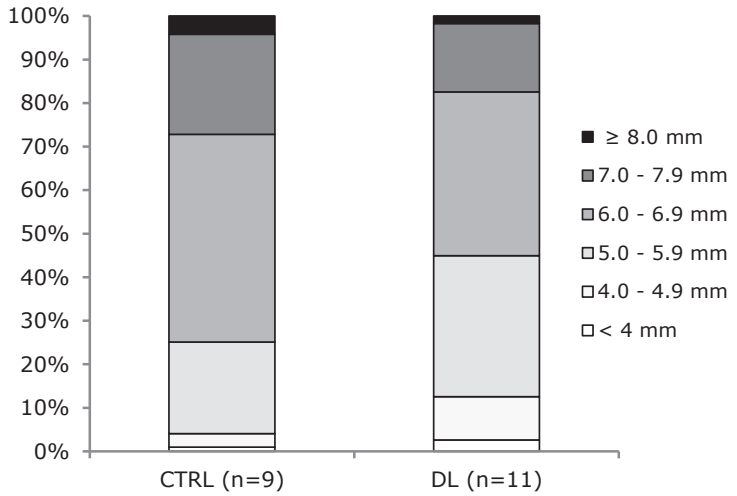

Figure 3.3 Percentage of follicles in each diameter category at day 4 after weaning, for sows fed either a dextrose and lactose- (each 150 g/day) containing diet at 4h intervals (DL) or an isocaloric control diet at 12h intervals (CTRL) during the WOI (from weaning until 12h after ovulation) (means).

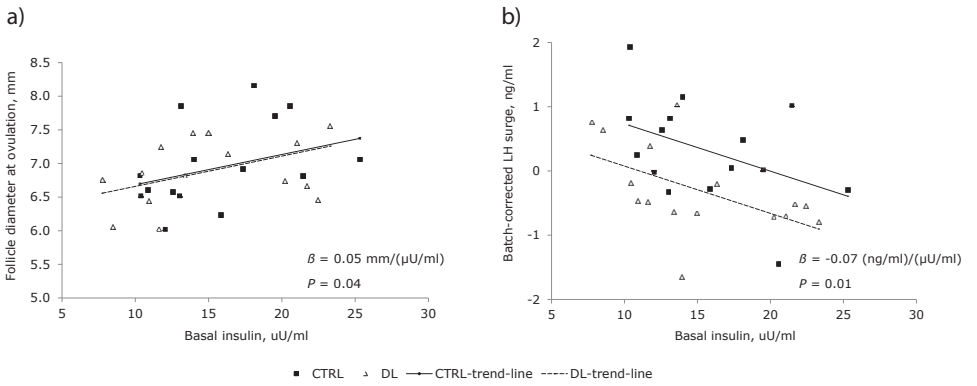

Figure 3.4 Relations between basal insulin level (mean days 2 and 3 after weaning) with **a)** follicle diameter at ovulation; and **b)** LH surge level (residuals corrected for the effect of batch; LSmeans were 2.96, 3.14 and 3.80 ng/ml for batch 1, 2 and 3 respectively; $P = 0.04$; absolute LH surge level was significantly higher in CTRL than in DL; 3.73 vs. 3.00 ng/ml; $P = 0.03$). The interactions between treatment and basal insulin level were not significant ($P > 0.10$); therefore overall treatment corrected regression coefficients are presented.

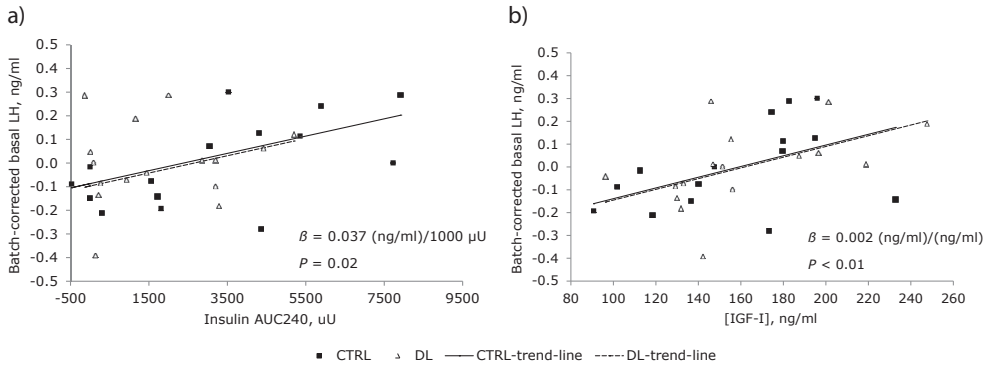

Figure 3.5 Relations between **a)** insulin AUC240 (mean days 2 and 3 after weaning); and **b)** IGF-1 level (mean level during days 3 - 5 after weaning) with basal LH level (residuals corrected for the effect of batch; LSmeans were 0.77, 0.98 and 1.03 ng/ml for batch 1, 2 and 3 respectively; $P = 0.01$). The interactions between treatment and insulin AUC240 or IGF-1 were not significant ($P > 0.10$); therefore overall treatment corrected regression coefficients are presented.

Insulin AUC240 ($ß = 0.037$ (ng/ml)/1000 µU; $P = 0.02$; Figure 3.5a) and mean IGF-1 level at days 3 - 5 after weaning ($ß = 0.002$ (ng/ml)/(ng/ml); $P < 0.01$; Figure 3.5b) were positively related to basal LH level. For CTRL sows, insulin AUC720 was also positively related to basal LH level ($ß = 0.030$ (ng/ml)/1000 µU; $P = 0.02$).

Insulin and IGF-1 parameters were not related to estrus and ovulation parameters, or the number, diameter and uniformity (SD; CV) of the follicle pool (≥ 3 mm) at day 4 after weaning.

Basal insulin and mean IGF-1 level at days 3 - 5 after weaning were correlated to each other ($r = 0.39$; $P = 0.03$), but not to insulin AUC240. Basal LH level and LH surge level were positively correlated to each other ($r = 0.38$; $P = 0.04$), but not to follicle diameter at ovulation.

DISCUSSION

Results of this study show that in multiparous sows, insulin and IGF-1 levels during the WOI are related to LH secretion and follicle development. Effects of insulin on LH and follicle development seem not only related to the absolute level of insulin and IGF-1, but also to the secretion pattern of insulin within a day, as indicated by a higher pre-ovulatory

LH surge and improved follicle development (larger and more uniform follicles) at day 4 after weaning in the CTRL sows compared to DL sows.

IGF-1 levels during the WOI were positively related to lactational backfat losses. In primiparous sows, high increases in IGF-1 levels after weaning are reported, especially in lactational feed restricted sows (Zak et al. 1998; Messias de Bragança and Prunier 1999; Van den Brand et al. 2001). In multiparous sows, IGF-1 levels and its postweaning increases are lower (Clowes et al., 1994; Langendijk et al., 2008), probably related to lower requirements for lean tissue growth. The positive relationship between lactational backfat loss and postweaning IGF-1 levels in our study may reflect a higher need for restoration of body stores in sows with high lactational backfat losses. However, lactational body weight and backfat losses were not related to follicle development, estrus or ovulation characteristics in our study.

The stimulating effect of insulin on pituitary LH release has been demonstrated *in vitro* (Adashi et al. 1981), and positive relationships between insulin and IGF-1 levels with LH secretion in lactating sows have been reported before (Tokach et al. 1992; Koketsu et al. 1996; Van den Brand et al. 2001). However, data on insulin and IGF-1 levels during the WOI is scarce. Paterson and Pearce (1994) measured mean insulin levels at days 1 and 3 after weaning in primiparous sows, but found no relationships between insulin levels and LH secretion.

In our study, basal insulin levels were related to follicle diameter at ovulation and LH surge level. Basal insulin levels are usually not influenced by feeding level, diet composition or feeding frequency (Kemp et al. 1995; Van den Brand et al. 1998, 2000; Zieçik et al. 2002), nor by physiological stages as pregnancy, lactation and weaning (Schaefer et al. 1991; Pere and Etienne 2007). This suggests that other factors, e.g. anticipatory neuro-physiological reflexes (Power and Schulkin 2008), determine basal insulin levels.

The importance of pre-ovulatory LH surge levels and basal LH levels around this LH surge for subsequent oocyte and embryo development are unknown. A suboptimal LH surge level may lead to inadequate luteinization of the corpora lutea, and consequently reduced plasma progesterone levels and increased embryo mortality (Einarsson and Rojkittikhun 1993).

The relationships between insulin and IGF-1 with follicle development and LH secretion were comparable for the two feeding regimens. Dextrose was used to stimulate a quick and high increase in insulin level directly after feeding (Van den Brand et al. 1998; Zieçik et al. 2002). Lactose was assumed to increase insulin levels for a prolonged period after

feeding, since adult sows have a reduced lactase activity in the small intestine (Kim et al. 1978), and consequently lactose was assumed to be fermented in the large intestine. The increase of lactobacilli concentrations in the large intestine after feeding lactose to finishing pigs found by Pierce et al. (2006), suggests that dietary lactose indeed increases fermentation activity. Fermentable carbohydrate sources (sugarbeet pulp) enhanced insulin levels for a prolonged period after feeding (Vestergaard 1997). Feeding two times a day results in higher and longer lasting insulin peaks, but also relatively long periods of low insulin levels. In an attempt to keep insulin levels enhanced and more constant for a prolonged period of the day, a DL diet was fed at a high feeding frequency (6 x /day). Because the DL sows were fed equal portions at 4h intervals, we expected the insulin responses to be similar after each meal. Therefore, we only measured insulin profiles after the 0800h feedings at days 2 and 3. If insulin AUC240 is multiplied by three to calculate AUC720 (as DL sows were fed three equal meals within 12h), insulin AUC720 for the DL sows would be 8569 μU on average, which is not different from the insulin AUC720 for the CTRL sows (7596 μU). In conclusion, the DL treatment (at a high feeding frequency and thus smaller meal sizes) neither resulted in a higher total insulin secretion (AUC720, mean insulin) nor in a more sustained period of increased insulin levels compared to the CTRL treatment.

The focus of this study was not on the separate effects of diet composition and feeding frequency. Nevertheless, both factors seem to interact regarding insulin secretion. In an additional experiment (Wientjes et al., 2012a), sows were fed the DL and CTRL diets in two equal portions per day (1.5 kg per portion), and the DL diet had a higher postprandial insulin AUC compared to the CTRL diet (3801 ± 326 vs. 2842 ± 310 μU/6.2h for the DL and CTRL diet, respectively). This indicates that when the diets are fed in two equal portions per day, the DL diet indeed results in a higher postprandial insulin response than the CTRL diet. In gilts, Zięzik et al. (2002) showed that a dextrose-rich diet fed 2 x /day or 3 x /day results in a significantly higher insulin AUC/220 min compared to a fat-rich diet, whereas at a feeding frequency of 4 x /day the postprandial insulin AUC/220 min was comparable for both diets. This indicates that at a higher feeding frequency (or smaller meal sizes), the effect of insulin-stimulating feed components (such as dextrose) decreases.

We conclude that increasing the feeding frequency, without increasing total daily feed intake, is not a proper way to stimulate total insulin secretion and to keep insulin at a more constant level during the day. However, by modulating both diet composition and feeding frequency, we created two completely different insulin secretion patterns. Besides

the insulin pattern, other metabolites or metabolic hormones (e.g. glucose, leptin, free fatty acids) are possibly affected by feed composition and feeding frequency. These were not studied here.

This study shows that the CTRL treatment had a beneficial effect on follicle development at day 4 after weaning (higher average diameter and uniformity, fewer small follicles) and LH secretion (higher pre-ovulatory LH surge) compared to the DL treatment. These follicles at day 4 after weaning represented the pre-ovulatory follicle pool, since the difference between number of follicles (≥ 3 mm) counted on day 4 after weaning and ovulation rate was only - 0.8 ± 0.4 (ranging from - 4 until 3). The treatment effects on follicle development and LH may be related to the secretion pattern of insulin. The DL sows reached peak insulin levels more frequently (6 x /day), but insulin levels rapidly dropped thereafter, whereas in CTRL sows peak levels were only reached twice a day, and insulin levels decreased only gradually thereafter. Insulin and IGF-1 can stimulate follicle and oocyte development via stimulation of LH (at brain level), but also directly at the ovarian level. For example, insulin can increase the number of LH receptors on granulosa cells (Poretsky and Kalin 1987), and could thereby stimulate follicles that have suboptimal numbers of LH receptors. Increasing responsiveness to LH of follicles could rescue them from atresia (which will result in a higher ovulation rate) and can further stimulate their development (which will result in improved average diameter and uniformity). In this context, also a higher ovulation rate in CTRL sows may be expected, which was indeed the case, although not significantly so (ovulation rate was 24.3 vs. 23.2 in CTRL and DL sows, respectively).

Thus, our results suggest that not only the total insulin secretion plays a role in follicle development and uniformity, but also the pattern of insulin secretion during the day may affect this process. These results further indicate that the positive effects of pre-mating insulin-stimulating diets on litter size, piglet birth weight and piglet uniformity (Van den Brand et al. 2009) may indeed be related to a stimulated and more uniform follicle development.

IMPLICATIONS

Absolute insulin and IGF-1 levels during the WOI showed relationships with LH secretion and follicle development in multiparous sows. Insulin secretion cannot be stimulated by an increased feeding frequency unless the total daily feed intake is increased.

Feeding strategies (feeding frequency and diet composition) which modulate the pattern of insulin secretion, even without affecting total daily insulin output, can influence LH secretion and development and uniformity of pre-ovulatory follicles. Our results suggest that two sustained insulin peaks per day (i.e. twice a day feeding) are more beneficial for follicle development and uniformity than frequent short insulin peaks per day (i.e. frequent feeding).

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Chapter 4

Nutritionally induced relationships between insulin levels during the weaning-to-ovulation interval and reproductive characteristics in multiparous sows: II. Luteal development, progesterone and conceptus development and uniformity

JGM Wientjes

NM Soede

H van den Brand

B Kemp

Adaptation Physiology Group,
Department of Animal Sciences,
Wageningen University,
Wageningen, The Netherlands

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ABSTRACT

Insulin-stimulating sow diets before mating improve piglet uniformity. We studied effects of nutritionally induced differences in insulin levels during the weaning-to-ovulation interval (WOI) on luteal development, progesterone secretion and pre-implantation conceptus development and uniformity (day 10). To create insulin contrasts, 32 multiparous sows were fed either a dextrose plus lactose-containing diet (each 150 g/day) at 4h intervals (DL treatment) or an isocalorically control diet (containing soybean oil) at 12h intervals (CTRL treatment) during the WOI. After ovulation, all sows received a standard pregnancy diet at 12h intervals. Ovulation rate, plasma progesterone levels, pregnancy rate and embryo survival did not differ between treatments. CTRL sows had a higher total luteal weight (11.2 vs. 9.7 g; $P = 0.03$) than DL sows. Conceptus diameter at day 10 of pregnancy tended to be larger in CTRL sows (diameter: 7.1 vs. 6.4 mm; $P = 0.07$). Conceptus uniformity was not influenced by treatment. Insulin area under the curve (AUC) and mean insulin during the WOI were positively related to mean progesterone (β-values were 0.78 (ng/ml)/1000μU and 0.14 (ng/ml)/(μU/ml) for AUC and mean, respectively; $P < 0.05$) and maximal progesterone (β-values were 1.46 (ng/ml)/1000μU and 0.27 (ng/ml)/(μU/ml) for AUC and mean, respectively; $P < 0.05$) levels during the first 10 days of pregnancy, but not to conceptus development and uniformity. In conclusion, high insulin levels during the WOI seem to be beneficial for progesterone secretion in sows, probably mediated through beneficial effects of insulin on follicle development.

INTRODUCTION

The increased litter size over the last decades is associated with increased pre-weaning piglet mortality, which is partly related to decreased litter uniformity (Milligan et al. 2002). Quesnel et al. (2008) concluded that sow factors as litter size, parity and season at conception together explained 20% of litter uniformity at birth, which indicates that a major part of the variation in litter uniformity is caused by other yet unknown factors.

Recent studies suggest that insulin-stimulating sow diets during the pre-mating period can influence uniformity in piglet development. Fermentable carbohydrates (50% sugarbeet pulp) in the pre-mating gilt diet improved uniformity in fetal weights at day 27 of pregnancy (Ferguson et al. 2006), and dextrose (150 g/day), either or not in combination with lactose (150 g/day), in the pre-mating sow diet (numerically) improved litter uniformity at birth (Van den Brand et al. 2006, 2009). The physiological mechanisms involved are unknown, but insulin and Insulin-like Growth Factor-1 (IGF-1) seem to be potential mediators. Insulin and IGF-1 are known to stimulate follicle and oocyte development, either indirectly at the brain level via stimulation of luteinizing hormone (LH; Koketsu et al. 1996; Van den Brand et al. 2001), or directly at the ovarian level (Poretsky and Kalin 1987; Quesnel et al. 2007).

As a first step to unravel the mechanism behind the effect of pre-mating sow diets on litter uniformity, we studied the effect of nutritionally induced differences in insulin secretion patterns, and corresponding IGF-1 levels, during the weaning-to-ovulation interval (WOI) on luteal development, progesterone secretion and embryo development and uniformity at day 10 of pregnancy.

MATERIALS AND METHODS

General design

During the WOI, multiparous (parity 5.9 ± 0.3; range 3 - 9) Topigs 20 (Topigs, Vught, The Netherlands) sows (n = 38) were fed either a dextrose plus lactose-containing diet at 4h intervals (DL treatment) or an isocaloric and isonitrogenous control diet (dextrose and lactose was exchanged by soybean oil) at 12h intervals (0800h and 2000h; CTRL treatment). Two factors, specific insulin-stimulating feed components (dextrose plus lactose) and a high feeding frequency (6 x /day), were used simultaneously to induce large contrasts in insulin

and IGF-1 levels and patterns among sows. After ovulation, all sows received a standard pregnancy diet at 12h intervals (0800h and 2000h) until slaughter at day 10 after ovulation.

Sows arrived at the experimental farm of Wageningen University within 2h from weaning (day 0), in three consecutive batches. At day of weaning, sows received either a permanent jugular vein catheter (23 sows) for blood sampling during the whole experimental period, or an ear vein catheter (15 sows) for blood sampling during the WOI. From day 2 after weaning, estrus detection was performed at 4h intervals by a back-pressure test in the presence of a boar. To determine time of ovulation, sows were transrectally scanned (Scanner 200; Pie Medical/Esaote, Maastricht, The Netherlands) at 12h intervals during estrus. Sows were inseminated every day of estrus with a commercial dose of semen (2 x 10^9 sperm cells) until ovulation had occurred.

Six sows (four CTRL, two DL) were veterinary treated for pneumonia (five sows) or diarrhea (one sow) during the experiment and were excluded from all analyses. All experimental procedures were approved by the Institutional Animal Use and Care Committee of Wageningen University (Wageningen, The Netherlands).

A complete description of animals used, animal management and diet compositions, and data on feed intake, insulin and IGF-1 profiles during WOI, follicle development, estrus and ovulation are reported elsewhere (Wientjes et al. 2012). After ovulation, all sows had a 100% feed intake. This paper focuses on effects on luteal development, progesterone, and conceptus development and uniformity.

Blood sampling

Frequent blood samples were taken around the 0800h feeding at day 2 and 3 after weaning for determination of glucose and insulin, and 0800h samples at day 1, 2, 3, 4 and 5 after weaning were analyzed for IGF-1, as described elsewhere (Wientjes et al. 2012). For sows with a jugular vein catheter, plasma samples taken at 4h intervals from 6h before until 18h after time of ovulation, and at 12h intervals thereafter until slaughter were analyzed for progesterone. For sows with an ear vein catheter, one blood sample was taken at slaughter at day 10 of pregnancy for determination of progesterone.

Blood samples with 100 µl EDTA (Tritiplex III, Merck Nederland B.V., Amsterdam, The Netherlands) solution (0.39M saline) were placed on ice, centrifuged at 1710 x g for 10 min at 4 °C, and plasma was stored at - 20 °C.

Uteri, ovaries and conceptuses

Sows were slaughtered at the experimental farm 9.5 or 10 days after ovulation, depending on time of ovulation. Immediately after stunning and exsanguination, reproductive tracts were removed, placed on ice and processed within 1h. Uterine horns and cervix were separated from the ovaries, oviducts and mesometrium. Both uterine horns were flushed twice with 30 ml 0.9% NaCl from the cervical to the ovarian end to collect the conceptuses. Conceptuses were immediately placed in 30 mL Dulbecco's PBS and kept on ice. The largest diameter (di1) and the largest diameter perpendicular to it (di2) of each conceptus (magnification of 1 x) and its embryoblast (magnification of 6.3 x) were measured using a stereo-microscope after spreading the conceptus as much as possible. Conceptuses were stored in 200 µL distilled water at - 20 °C, thawed and frozen five times and sonificated (2 x 10 pulses; Branson Sonifier 250, Boom BV, Meppel, The Netherlands) until further analyses.

For diameter analyses the largest diameter (di1) was used. Conceptus surface area was calculated as: $2\pi ab$, where $a = {}^1/_2$ di1; and $b = {}^1/_2$ di2. When no conceptuses were recovered, sows were considered non-pregnant (n = 3, including one sow (DL) that developed cystic ovaries). Additionally, in one sow (DL) with turbid uterine flushings, all conceptuses (n = 10) were non-vital, based on abnormal morphological appearance (abnormal dark/brown color and shriveled up). Other parameters (ovulation rate, luteal development and progesterone levels) seemed normal, and therefore we considered this sow as pregnant at day 10 of pregnancy. Furthermore, one sow (DL) was not inseminated due to silent estrus.

Corpora lutea were counted. Individual corpora lutea were dissected and diameter, weight and quality (normal, hemorrhagic, cystic) was determined. Total luteal weight was calculated as the sum of weights of the individual corpora lutea. Embryo survival was defined as the number of conceptuses divided by the number of corpora lutea.

Plasma analyses

Analysis of plasma glucose, insulin and IGF-1 levels, and a complete definition of glucose, insulin and IGF-1 parameters used, are described elsewhere (Wientjes et al. 2012). For each sampling day, basal insulin levels were calculated as the mean value of the two samples taken before feeding (- 12 and 0 min); AUC240 (area under the curve during a 240 min period) was calculated as the area above basal insulin levels from feeding until 240 min after feeding for all sows; only for CTRL sows AUC720 (area under the curve during a 720 min period) was calculated; and the mean insulin level during the sampling period was

calculated as the average insulin levels of all plasma samples after feeding (from 0 until 720 min in CTRL; from 0 until 240 min in DL) corrected for the time intervals between samples.

Progesterone levels were determined in duplicate, using a commercial Coat-A-Count Progesterone RIA-kit (PITKPG-7®; Siemens Medical Solutions Diagnostics, Los Angeles, CA, USA). The sensitivity, intra- and interassay CV were 0.1 ng/ml, 4.7% and 6.0%, respectively. Basal progesterone levels were calculated as the average value of the first two samples (6h and 2h before ovulation); maximal progesterone levels were calculated as the average value of the last two samples before slaughter. Mean progesterone levels were calculated as the average progesterone level of all plasma samples, corrected for the time intervals between samples. For sows with an ear vein catheter, maximal progesterone level was defined as progesterone level at slaughter.

Conceptus analyses

Protein content of conceptuses was analyzed in duplicate with the method as described by Bradford (1976), using the microplate-procedure of a commercial kit (QuickStart Bradford Protein Assay®; Bio-Rad, Hercules, CA, USA), with Bovine Serum Albumin as standard. DNA content of conceptuses was measured fluorometrically in duplicate using a commercial kit (Quant-iT dsDNA Assay Kit, Broad Range Q33130®; Invitrogen, Ltd, Paisley, UK).

Statistical analyses

Data are presented as means ± SE. Analysis of variance was applied to continuous data using the GLM procedure of SAS 9.1 (SAS Inst. Inc., Cary, NC, USA). The factors and interactions included in the statistical model were tested for significance and stepwise omitted from the model if $P > 0.10$ (except for the factor treatment), using Bonferroni corrections for multiple comparisons. Pearson correlation was used to analyze relationships among continuous data. When luteal and conceptus characteristics were significantly affected by batch and/or age of the conceptuses, batch and/or conceptus-age corrected residuals were used for calculation of correlations. Analysis of feed intake, insulin and IGF-1 levels during WOI is described elsewhere (Wientjes et al. 2012).

One sow (DL) developed cystic ovaries (ovulation rate: 3) and was excluded from analyses on luteal development and progesterone. Luteal development and plasma progesterone levels of remaining non-pregnant sows (n = 3) and the sow with non-vital embryos seemed

normal and were therefore included in all analyses. Two sows (both CTRL) had cystic corpora lutea on both ovaries (10 of 22 and 4 of 24 corpora lutea) and were excluded from analyses on total luteal weight, corpus luteum diameter and weight. The sow with non-vital embryos was excluded from analyses on number of conceptuses, embryo survival and conceptus development and uniformity. Two sows (one CTRL, one DL) contained filamentous conceptuses and were excluded from all analyses on conceptus development and uniformity. The statistical model for luteal development and progesterone parameters, and for number of conceptuses and embryo survival rate, included treatment (CTRL, DL), batch (1,2,3), DMI_{WOI} (DMI from weaning until 12h after ovulation; dietary treatment period) in classes (where 0 = < 75% and 1 = ≥ 75%), and the interaction between treatment and DMI_{WOI}. For conceptus characteristics and uniformity, age of the conceptuses (9.5 or 10 days) was added as additional factor.

Differences in numbers of sows being pregnant between treatments were analyzed using the Chi-square test of SAS 9.1.

In all these analyses, relevant covariables and their interaction with treatment (if relevant) were added, e.g. to analyze whether luteal and conceptus development were related to follicle development, to analyze relations between insulin and IGF-1 parameters with reproductive parameters, or to analyze effects of body weight losses and backfat losses during lactation on subsequent luteal and conceptus parameters.

RESULTS

Luteal development and progesterone

Total luteal weight was higher in CTRL sows (+ 1.5 g; $P = 0.03$), and mean corpus luteum diameter and weight tended to be higher in CTRL (+ 0.4 mm ($P = 0.06$) and + 0.05 g ($P = 0.09$), respectively) than in DL sows (Table 4.1). The effect of treatment on corpus luteum diameter and weight disappeared when average diameter of follicles ≥ 3 mm at day 4 after weaning was added to the models for corpus luteum diameter (*ß* for follicle diameter was 1.23 mm/mm; $P = 0.03$) and weight (*ß* for follicle diameter was 0.06 g/mm, $P = 0.07$).

Plasma progesterone levels were comparable between treatments (Table 4.1). Mean progesterone was positively affected by DMI_{WOI} (LSmeans were 13.60 and 16.31 ng/ml for sows with a low (< 75%) and high (≥ 75%) DMI_{WOI}, respectively; $P = 0.05$).

Table 4.1 Luteal development and progesterone characteristics for sows fed either a dextrose plus lactose (each 150 g/day) containing diet at 4h intervals (DL) or an isocaloric control diet at 12h intervals (CTRL) during the WOI (from weaning until 12h after ovulation) (means ± SE)

	Treatment		*P*-value[1]	
Item	CTRL	DL	Treatment	DMI_{WOI}[2]
Number of sows	15	16[3]		
Luteal development				
Ovulation rate	24.3 ± 1.2	23.2 ± 0.8	0.43	-
Total luteal weight[4], g	11.2 ± 0.5	9.7 ± 0.5	0.03	-
Mean corpus luteum diameter[4], mm	10.0 ± 0.3	9.6 ± 0.3	0.06	< 0.01[6]
Mean corpus luteum weight[4], g	0.47 ± 0.02	0.42 ± 0.02	0.09	-
Progesterone				
Basal progesterone[5], ng/ml	0.49 ± 0.07	0.86 ± 0.21	0.23	0.06[6]
Mean progesterone[5], ng/ml	14.60 ± 1.35	14.70 ± 0.90	0.96	0.05[6]
Maximal progesterone, ng/ml	30.57 ± 2.14	29.71 ± 1.91	0.28	-

[1] Statistical significance; the treatment * DMI_{WOI} interactions were never significant; - when not significant ($P > 0.10$), factors were removed from the model (except treatment).
[2] DMI from weaning until 12h after ovulation, as % of total dry matter offered (< 75%, ≥ 75%).
[3] Additionally, one DL sow developed cystic ovaries.
[4] Additionally, two CTRL sows had cystic corpora lutea on both ovaries.
[5] Only for sows with a jugular vein catheter (n = 10 CTRL and 11 DL sows).
[6] For sows with a low (< 75%) and high (≥ 75%) DMI_{WOI} respectively, LS means were 10.4 and 9.4 mm for mean corpus luteum diameter, 0.89 and 0.45 ng/ml for basal progesterone, and 13.60 and 16.31 ng/ml for mean progesterone.

Conceptus development and uniformity

A total of 28 sows were pregnant at slaughter and pregnancy rate did not differ between treatments (93% in CTRL; 88% in DL, $P = 0.58$).

Conceptus diameter tended to be higher in CTRL than in DL sows (+ 0.7 mm; $P = 0.07$), but other conceptus characteristics and conceptus uniformity did not differ between treatments (Table 4.2). Mean conceptus diameter and conceptus protein content were not related to follicle diameter at day 4 after weaning or at ovulation.

Relationships between insulin and IGF-1 with reproduction parameters

In the analyses, the interaction between treatment and insulin parameters (mean values of days 2 and 3) or IGF-1 level (mean value of days 3 - 5 after weaning) was for none of the reproductive parameters significant ($P > 0.10$), indicating that relationships were

Table 4.2 Conceptus development and uniformity at day 10 of pregnancy for sows fed either a dextrose plus lactose (each 150 g/day) containing diet at 4h intervals (DL) or an isocaloric control diet at 12h intervals (CTRL) during the WOI (from weaning until 12h after ovulation) (means ± SE)

	Treatment		*P*-value[1]	
Item	CTRL	DL	Treatment	DMI_{WOI} [2]
Number of pregnant sows	14[3]	14[3]		
Number of conceptuses[4]	21.9 ± 1.1	20.2 ± 0.9	0.27	-
Embryo survival[4], %	90 ± 2	88 ± 2	0.99	-
Diameter[4,5,6], mm	7.1 ± 0.47	6.4 ± 0.64	0.07	-
SD[4,5,6], mm	1.2 ± 0.14	1.2 ± 0.14	0.65	-
CV[4,5], %	17.4 ± 1.64	18.8 ± 2.14	0.60	-
Surface area[4,5,6], mm^2	73.0 ± 10.5	63.4 ± 11.2	0.11	-
SD[4,5,6], mm^2	20.3 ± 2.5	19.0 ± 3.0	0.20	-
CV[4,5], %	28.6 ± 1.9	31.4 ± 2.8	0.40	-
Embryoblast diameter[4,5,6], mm	0.43 ± 0.02	0.40 ± 0.03	0.20	-
SD[4,5,6], mm	0.07 ± 0.01	0.07 ± 0.01	0.77	-
CV[4,5], %	17.5 ± 1.2	18.2 ± 1.5	0.72	-
Protein content[4,5,6], µg	86 ± 9	75 ± 11	0.29	-
SD[4,5,6], µg	26 ± 2	23 ± 3	0.23	-
CV[4,5], %	32 ± 2	32 ± 3	0.88	-
DNA content[4,5,6], ng	349 ± 33	329 ± 36	0.32	-
SD[4,5,6], ng	169 ± 14	138 ± 15	0.08	-
CV[4,5], %	44 ± 9	46 ± 5	0.88	-

[1] Statistical significance; the treatment * DMI interactions were never significant; - when not significant ($P > 0.10$), factors were removed from the model (except treatment).
[2] DMI from weaning until 12h after ovulation, as % of total dry matter offered (< 75%, ≥ 75%).
[3] Additionally, one DL sow had silent estrus, one DL sow developed cystic ovaries and in two sows (one CTRL and one DL) no conceptuses were recovered.
[4] One sow (DL) with non-vital conceptuses was excluded.
[5] Two sows (one CTRL and one DL) with filamentous conceptuses were excluded.
[6] Corrected for significant effect ($P < 0.05$) of age of conceptuses (9.5 or 10 days).

comparable for both treatments. Therefore overall treatment corrected regressions are presented. An overview of significant relationships ($P < 0.05$) is given in Table 4.3.

Insulin parameters were not related to ovulation rate, total luteal weight and corpus luteum weight. Insulin AUC240 and mean insulin were negatively related to mean corpus luteum diameter (Table 4.3 & Figure 4.1). For CTRL sows, insulin AUC720 was negatively related to mean corpus luteum diameter (β = - 0.23 mm/1000 µU; $P = 0.01$) and weight (β = - 0.11 g/1000 µU; $P = 0.02$).

Table 4.3 Relations between insulin parameters (mean values of days 2 and 3 after weaning) and luteal development and progesterone parameters ($P < 0.05$)

	Insulin parameters[1]	
Luteal development and progesterone	Insulin AUC240, µU	Mean insulin, µU/ml
Mean corpus luteum diameter, mm	ß = - 0.22 mm/1000 µU $P = 0.02$	ß = - 0.06 mm/(µU/ml) $P = 0.01$
Mean progesterone, ng/ml	ß = 0.78 (ng/ml)/1000 µU $P = 0.01$	ß = 0.14 (ng/ml)/(µU/ml) $P = 0.05$
Maximal progesterone, ng/ml	ß = 1.46 (ng/ml)/1000 µU $P < 0.01$	ß = 0.27 (ng/ml)/(µU/ml) $P = 0.05$

Overall treatment-corrected regressions ($P < 0.05$) are presented; when significant batch- and/or conceptus-age-effects existed ($P < 0.10$), regressions were corrected for these effects.
[1] Mean value of days 2 and 3 after weaning.

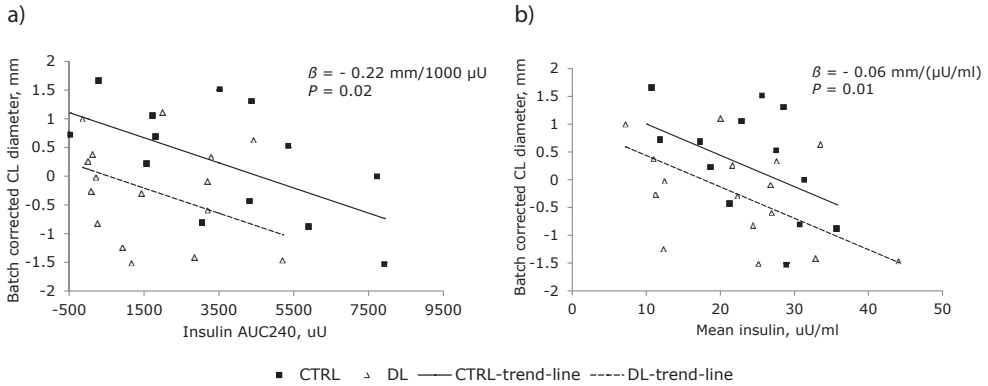

Figure 4.1 Relations between **a)** insulin AUC240 (mean days 2 and 3 after weaning); and **b)** mean insulin level (mean days 2 and 3 after weaning) with corpus luteum diameter (residuals corrected for the effect of batch; LSmeans were 10.5, 9.6 and 9.6 mm for batch 1, 2 and 3 respectively; $P = 0.04$; corpus luteum diameter tended to be higher in CTRL than in DL; 10.0 vs. 9.6 mm; $P = 0.06$). The interactions between treatment and insulin AUC240 or mean insulin were not significant ($P > 0.10$); therefore, overall treatment corrected regression coefficients are presented.

A positive relationship existed between both insulin AUC240 and mean insulin with mean progesterone levels during the first 10 days of pregnancy (Table 4.3 & Figure 4.2a), as well as with maximal progesterone levels at day 10 of pregnancy (Table 4.3 & Figure 4.2b). For CTRL sows, insulin AUC720 was negatively related to basal progesterone (ß = - 0.03 (ng/ml)/1000 µU; $P = 0.01$), but positively related to mean progesterone (ß = 0.51 (ng/ml)/1000 µU; $P < 0.01$) and maximal progesterone (ß = 1.20 (ng/ml)/1000 µU; $P < 0.01$).

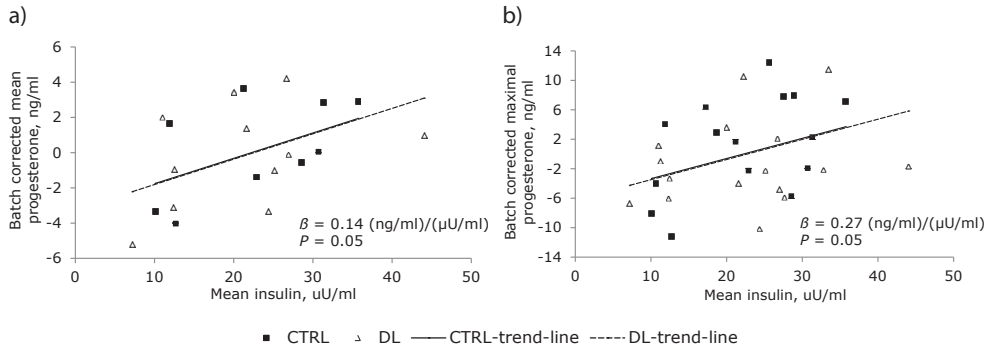

Figure 4.2 Relations between mean insulin (mean days 2 and 3 after weaning) with **a)** mean progesterone level (residuals corrected for the effect of batch; LSmeans were 18.14, 13.92 and 12.80 ng/ml for batch 1, 2 and 3 respectively; $P < 0.01$); and **b)** maximal progesterone level at day 10 of pregnancy (residuals corrected for the effect of batch; LSmeans were 37.47, 28.84 and 26.04 ng/ml for batch 1, 2 and 3 respectively; $P < 0.01$). The interactions between treatment and mean insulin were not significant ($P > 0.10$); therefore, overall treatment corrected regression coefficients are presented.

Mean IGF-1 during days 3 - 5 after weaning was not related to luteal development and progesterone levels. Insulin and IGF-1 parameters were not related to number of conceptuses, embryo survival, and conceptus development and uniformity.

Insulin AUC240 and mean insulin were highly correlated ($r = 0.79$; $P < 0.0001$). Mean and maximal progesterone were also highly correlated ($r = 0.87$; $P < 0.0001$).

DISCUSSION

This study was designed to investigate the mechanism by which pre-mating sow diets affect litter uniformity. We hypothesized that (nutritionally induced) stimulation of insulin and/or IGF-1 release would result in a more developed and a more uniform pre-ovulatory follicle pool (see Wientjes et al. 2012), resulting in further developed and more uniform embryos and improved luteal development, finally resulting in more uniform birth weights. Results of this study partly confirm our hypothesis. Positive relationships were found between pre-ovulatory insulin levels and (i) LH secretion and follicle diameter (Wientjes et al. 2012); and (ii) progesterone levels during the first 10 days of pregnancy. Furthermore, follicle size was reflected in size of the corpora lutea. However, pre-ovulatory insulin and IGF-1 levels were not related to development and uniformity of conceptuses at

day 10 of pregnancy in this study. After day 10 of pregnancy, conceptuses start to elongate and the implantation process starts. During this peri-implantation period, part of the embryos will be lost (Pope and First 1985). Additionally, conceptus development at day 10 of pregnancy is a highly variable trait, due to the rapid development of conceptuses at this stage. Therefore, it is possible that development and uniformity of day 10 conceptuses is not a reliable predictor for piglet uniformity at birth, and it seems likely that effects of pre-mating insulin levels on piglet uniformity are not yet visible at this early stage of pregnancy.

Results of this study showed that in multiparous sows, insulin levels (mean and AUC) during the WOI are positively related to both mean and maximal progesterone level during the first 10 days of pregnancy. This is most probably mediated through effects of insulin on follicle development. Insulin AUC at days 2 and 3 after weaning was positively related to basal LH levels around the LH surge, and basal insulin levels at days 2 and 3 after weaning were positively related to follicle size at ovulation (Wientjes et al. 2012). We further found positive relations between mean follicle diameter at day 4 after weaning and mean corpus luteum diameter and weight, indicating that larger follicles develop into larger corpora lutea. Similarly, Soede et al. (1998) found a positive relationship between average follicle volume at ovulation (as measured by ultrasound) and average corpus luteum weight ($r = 0.28$; $P < 0.01$) at day 5 of pregnancy in sows. More evidence for a direct relationship between pre-ovulatory follicle development and subsequent luteal development and progesterone secretion comes from studies in cows (Vasconcelos et al. 2001; Echternkamp et al. 2009). Larger corpora lutea have a higher luteal weight, and total luteal weight was strongly correlated to maximal plasma progesterone level at day 10 of pregnancy ($r = 0.51$; $P < 0.01$) in this study. Therefore, we conclude that an increased total luteal weight, as a result of either a higher ovulation rate or larger individual corpora lutea or both, seems to be beneficial for progesterone production and secretion in sows.

Pre-mating insulin levels were not significantly related to conceptus development or uniformity at day 10 of pregnancy in this study. However, progesterone level and conceptus development were highly correlated ($r = 0.44$; $P = 0.03$). Furthermore, insulin AUC240 tended to be positively related to conceptus DNA content ($ß = 16.38$ ng/1000 µU; $P = 0.07$; data not shown) and mean insulin tended to be positively related to conceptus diameter ($ß = 0.06$ mm/(µU/ml); $P = 0.09$; data not shown). The correlation between progesterone level and conceptus development may be causal. Progesterone regulates quantitative and

qualitative changes in uterine protein secretion (Knight et al. 1973; Stroband and Van der Lende 1990; Davis and Blair 1993; Vallet et al. 1998), and progesterone treatments at days 2 and 3 of pregnancy have been shown to increase total uterine protein at days 10 - 15 and advance conceptus estrogen production at day 11 of pregnancy (Vallet et al. 1998; Vallet and Christenson 2004). On the other hand, Ashworth et al. (1999a, b) showed that pre-mating nutrition can affect embryo survival and development at day 12 of pregnancy without significant changes in plasma progesterone levels and uterine fluid composition. This could indicate that the correlation between progesterone and conceptus development is not (completely) causal, but they may also share a common origin in the pre-mating period, e.g. in follicle and oocyte development.

The CTRL treatment resulted in larger corpora lutea (related to larger pre-ovulatory follicles) and further developed conceptuses compared to the DL treatment. These beneficial effects of the CTRL treatment might be related to the secretion pattern of insulin (related to feeding frequency and meal sizes), and suggests that not only the total amount of insulin secreted, but also the pattern of insulin secretion during the day may affect follicle development and subsequent luteal and conceptus development.

Ferguson et al. (2004, 2006, 2007) reported that high fibre diets prior to ovulation, which improved oocyte maturation (Ferguson et al. 2007), are beneficial for survival and uniformity of fetuses at day 27 of pregnancy (Ferguson et al. 2006), and result in a higher number of piglets born (11.5 vs. 10.9 piglets born alive compared to an isocaloric control diet; Ferguson et al. 2004). Van den Brand et al. (2009) showed that dextrose plus lactose in the diet prior to ovulation not only numerically improved uniformity in birth weights (- 3% in birth weight CV), but also numerically increased litter size (13.5 vs. 13.0 piglets born alive compared to an isocaloric control diet); the lack of significance in their study is probably related to the experimental setup, in which stable and not sow had to be used as the experimental unit. This suggests that pre-mating insulin-stimulating diets not only improve litter uniformity, but may also improve embryo survival. The improved embryo survival may be (partly) due to beneficial effects of pre-mating insulin-stimulating diets on progesterone levels (as found in the current study), since plasma progesterone levels during early pregnancy are related to embryo survival (Ashworth 1991; Jindal et al. 1996, 1997; Van den Brand et al. 2000). Whether and how the improved progesterone production during early pregnancy results in more uniform development of fetuses and piglets needs further study.

IMPLICATIONS

Insulin levels during the WOI were not related to development and uniformity of pre-implantation conceptuses. However, positive relationships were found between insulin levels during the WOI and plasma progesterone levels during the first 10 days of pregnancy. This effect is most probably mediated through stimulatory effects of insulin on follicle development, which is reflected in luteal development. Improved progesterone levels might be beneficial for subsequent survival, development and uniformity of embryos and fetuses at later stages of pregnancy.

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Chapter 5

Insulin-stimulating diets during the weaning-to-estrus interval do not improve fetal and placental development and uniformity in high prolific multiparous sows

JGM Wientjes
NM Soede
BFA Laurenssen
RE Koopmanschap
H van den Brand
B Kemp

Adaptation Physiology Group,
Department of Animal Sciences,
Wageningen University,
Wageningen, The Netherlands

Submitted for publication to ANIMAL

ABSTRACT

Piglet birth weight and litter uniformity are important for piglet survival. Insulin-stimulating sow diets before mating may improve subsequent piglet birth weights and litter uniformity, but the physiological mechanisms involved are not clear. This study evaluated effects of different levels of insulin-stimulating feed components (dextrose plus starch; fed twice daily) during the weaning-to-estrus interval (WEI) on plasma insulin and IGF-1 concentrations, and on follicle development and subsequent luteal, fetal and placental development and uniformity at day 42 - 43 of pregnancy. During WEI, multiparous sows were isocalorically fed diets supplemented with 375 g/day dextrose plus 375 g/day corn starch (INS-H), with 172 g/day dextrose plus 172 g/day corn starch and 144 g/day animal fat (INS-L), or with 263 g/day animal fat (CON). Jugular vein catheters were inserted through the ear vein at 1.5 days before weaning to asses plasma insulin and IGF-1 concentrations. After estrus, all sows received a standard pregnancy diet until slaughter at day 42 - 43 of pregnancy. The dextrose plus starch-diets enhanced the postprandial insulin response in a dose-dependent manner (e.g. at day 2 insulin area under the curve was 4516 μU/444 min for CON, 8197 μU/444 min for INS-L, and 10894 μU/444 min for INS-H, SEM = 694, $P < 0.001$), but did not affect plasma IGF-1 concentrations during the first 3 days of WEI. Follicle development and subsequent luteal, fetal and placental development and uniformity were not affected by the dietary treatments, nor related to plasma insulin and IGF-1 concentrations during WEI. Pre-weaning plasma insulin and IGF-1 concentrations were negatively related to sow body condition loss during lactation, but were not related to subsequent reproduction characteristics. This study shows that dietary dextrose plus starch are effective in stimulating insulin secretion (both postprandial peak and long term concentration), but not IGF-1 secretion during the first 3 days after weaning in multiparous sows. The extreme insulin-stimulating diets during WEI did, however, not improve follicle development, or subsequent development and uniformity of fetuses and placentas in these high prolific sows (27.0 ± 0.6 ovulations; 18.6 ± 0.6 vital fetuses).

INTRODUCTION

Important factors for pre-weaning piglet survival are piglet birth weight and litter uniformity (Milligan et al. 2002; Quiniou et al. 2002). Higher piglet birth weights and litter uniformity have been found using insulin-stimulating sow diets before mating (Van den Brand et al. 2006, 2009), probably through beneficial effects of insulin and/or IGF-1 on follicle development [as reviewed by Quesnel (2009)]. In a previous study, we found positive relationships between pre-mating insulin concentrations and LH, follicle development, and subsequent progesterone concentrations (luteal development) and embryo size (but not uniformity) at day 10 of pregnancy (Wientjes et al. 2012b, c). Whether and how these effects lead to a more uniform development of fetuses at later stages of pregnancy, however, needs further study.

In these previous experiments with pre-mating diets (Van den Brand et al. 2006, 2009; Wientjes et al. 2012b, c), dextrose and/or lactose were used as the insulin-stimulating feed components, always at a level of 150 g/day. Effects on litter uniformity, however, may depend on the level of insulin-stimulating feed components and accompanying plasma insulin concentrations. Two sustained insulin peaks per day (i.e. twice a day feeding) seem to be more beneficial for follicle development and subsequent luteal and embryo development than a similar amount of insulin secreted in frequent short insulin peaks per day [i.e. frequent feeding; Wientjes et al. (2012b, c)]. Focus of this study is, therefore, on relations with insulin profiles using twice a day feeding. Further, high and sustained insulin peaks may be more effectively obtained using dietary supplementation with dextrose plus starch than supplementation with only starch (Van den Brand et al. 1998) or dextrose plus lactose (Wientjes et al. 2012a).

The main objective of this study was to evaluate effects of different levels of insulin-stimulating feed components (dextrose plus starch; fed twice daily) during the weaning-to-estrus interval (WEI) on plasma insulin and IGF-1 concentrations, and on follicle development and subsequent luteal, fetal and placental development and uniformity at day 42 - 43 of pregnancy in multiparous sows. Additionally, we aim to study whether and how these reproduction characteristics are related to plasma insulin and IGF-1 concentrations.

MATERIALS AND METHODS

General design

During WEI, multiparous sows were isocalorically fed a diet supplemented with different levels of dextrose plus corn starch and/or animal fat. After estrus, all sows received a standard pregnancy diet until slaughter at day 42 - 43 of pregnancy, after which luteal, fetal and placental development was assessed. All experimental procedures were approved by the Institutional Animal Use and Care Committee of Wageningen University (Wageningen, The Netherlands).

Animals and management

General management

Throughout the experiment, sows were fed twice daily (at 0730h and 1530h), feed refusals were removed within 1h after feeding (at 36 min after feeding on blood sampling days), and water was available *ad libitum*. During WEI and on blood sampling days, feed refusals were weighed, and refusal samples were stored at 4 °C until analysis of dry matter content. During pregnancy and lactation, feed refusals were recorded as none (< 10%), moderate (10 - 50%) or high (> 50%). Sows were exposed to 12h of light (0700 - 1900h) during pregnancy and lactation, and 16h of light (0700 - 2300h) during WEI. Room temperature was kept at 18 °C during pregnancy and WEI, 24 °C around farrowing and 19 °C during lactation.

Previous pregnancy and lactation

At day 35 of pregnancy, 60 multiparous (parity 4.7 ± 1.1 at farrowing) Topigs 20 (Topigs, Vught, The Netherlands) sows, from one farm, arrived at the experimental farm of Wageningen University in three consecutive batches of 20 sows. During pregnancy, sows were housed in groups of five sows in pens with individual feeding stalls, and fed a standard pregnancy feed (8.5 MJ NE/kg, 134 g/kg CP, 5.6 g/kg ileal digestible lysine) at a level of 2.8 kg/day (day 35 to 84) or 3.1 kg/day (day 85 to 108). From day 108 of pregnancy, sows were crated in farrowing pens, and were gradually switched to a standard lactation feed (9.0 MJ NE/kg, 155 g/kg CP, 7.7 g/kg ileal digestible lysine) at a level of 2.8 kg/day. On the day of farrowing, sows received 1.5 kg feed. Thereafter, feed allowance was gradually increased to a maximum of 7 kg/day at day 17 of lactation. Piglets were fed a creep feed from 3 days of age onwards. Sows farrowed 17.3 ± 0.4 piglets (14.7 ± 0.5 liveborn and 2.5

± 0.4 stillborn). Litter sizes were standardized within 3 days after birth. At 1.5 day before weaning (11.3 ± 0.3 piglets weaned), 57 sows received a jugular vein catheter through the ear vein as described by Wientjes et al. (2012b). The remaining three sows were excluded from the experiment due to farrowing problems (n = 1), severe lameness (n = 1) or diarrhea and low feed intake during a large part of lactation (n = 1).

Weaning-to-estrus interval

After weaning at 26.0 ± 0.2 days of lactation (at 1530h; day 0), sows were crated in feeding stalls, and fed 1 kg of the lactation feed. Thereafter, 57 sows were assigned to the experimental diets (fed until end of estrus), based on parity class (≤ 4 or ≥ 5 at previous farrowing) and body weight loss during lactation (%). Sows received either a diet supplemented with 375 g/day dextrose plus 375 g/day corn starch (INS-H; n = 16) or with 172 g/day dextrose plus 172 g/day corn starch (INS-L; n = 18), or received a control diet (CON; n = 23). Therefore, dextrose plus corn starch (both 375 g/day; INS-H) or animal fat (263 g/day; CON) were added to a basal diet containing sufficient protein, vitamins and minerals (Table 5.1; manufactured by Research Diet Services BV, Wijk bij Duurstede, The Netherlands). The INS-L diet was a mixture of the INS-H and CON diet (1:1). With daily feed allowances of 3.0 kg for INS-H, 2.75 kg for INS-L and 2.5 kg for CON, all diets were fed to be isocaloric and isonitrogenous. From 1.5 days after weaning, estrus detection was performed at 8h intervals (at 0800h, 1600h and 2400h) by a back-pressure test in the presence of a mature boar. Sows were inseminated every day of estrus (at 1600h) with a commercial available dose of semen (2 x 10^9 sperm cells) of a Topigs boar line. Ear vein catheters were removed at 3 days after weaning.

Pregnancy

After end of estrus until slaughter at day 42 - 43 of pregnancy, sows were group housed and fed 2.8 kg/day of the standard pregnancy diet (as in previous pregnancy). At 18 - 25 days after insemination, sows were checked for estrus once daily using a mature boar, and at 25 days after insemination pregnancy was determined by transcutaneous ultrasonography.

Table 5.1 Composition of the experimental diets (as fed)

Ingredient	INS-H, g		INS-L, g		CON, g	
Wheat	149.5		150.7		149.5	
Barley	150.0		151.2		150.0	
Wheat middlings	120.0		120.9		120.0	
Sugarbeet pulp (sugar < 100 g/kg)	75.0		75.6		75.0	
Soybean meal, extracted (CF < 50 g/kg)	75.0		75.6		75.0	
Sunflower seed, extracted (CF 200 - 240 g/kg)	75.0		75.6		75.0	
Rape seed, extracted (CP < 380 g/kg)	75.0		75.6		75.0	
Animal fat	10.0		10.0		10.0	
Additional animal fat	0		48.1		87.7	
Dextrose	125.0		57.4		0	
Corn starch	125.0		57.4		0	
Vitamin-mineral premix	5.0		5.0		5.0	
Limestone	8.5		8.6		8.5	
Monocalciumphosphate	4.0		4.0		4.0	
Salt	3.0		3.0		3.0	
Total, g[1]	**1000**		**919**		**838**	
	Calc.[4]	Anal.	Calc.	Anal.	Calc.	Anal.
Content	g/1000 g		g/919 g		g/838 g	
Dry matter	883.0	887.4	814.8	823.8	746.0	754.3
Crude fat	24.7	28.7	72.3	75.3	111.2	113.4
Crude protein	141.3	140.9	142.1	141.0	140.6	139.2
Starch[2]	303.9	268.2	249.1	207.8	199.7	161.9
Sugar	153.1	145.6	91.2	88.3	38.1	42.4
MJ NE (for swine)[3]	8.98	-	9.04	-	8.97	-

[1] 1000 g of the INS-H diet, 919 g of the INS-L diet and 838 g of the CON diet are isocaloric and isonitrogenous.
[2] Calculated: Ewers method; analyzed: enzymatic method.
[3] According to the Centraal Veevoederbureau (CVB 2003).
[4] Calc. = calculated; Anal. = analyzed.

Measurements

Sow body condition

Sows were weighed and P2 backfat thickness was measured within 24h after farrowing and at weaning. Sow body weight loss (and backfat loss) during lactation was calculated as body weight (or backfat thickness) at farrowing minus body weight (or backfat thickness) at weaning.

Follicle development and estrus

On day 0.5 and day 4.5 after weaning, follicle diameter was determined with transrectal ultrasonography (Scanner 200; Pie Medical/Esaote, Maastricht, The Netherlands), by averaging the diameter of the five largest follicles at one ovary. Time of onset of estrus was defined as 4h before the first time a sow showed a standing response; end of estrus was defined as 4h after the last time the sow showed a standing response. Ovulation was estimated to occur at 70% of the way through estrus (Soede and Kemp 1997).

Luteal, fetal and placental development

Non-pregnant sows (including one CON sow that aborted on day 27 of pregnancy) were slaughtered to determine the number of corpora albicantia. Pregnant sows were slaughtered at 42 or 43 days after estimated time of ovulation. Immediately after stunning and exsanguination, reproductive tracts were removed and placed on ice. Ovaries were removed and individual corpora lutea were counted, dissected and weighed. Total luteal weight was calculated as the sum of weights of the individual corpora lutea. After removal of the mesometrium and separation of uterine horns, the horns were cut open at the antimesometrial side, and number of total and vital fetuses was counted (vitality was based on size and color). After separating fetuses and placentas, length, weight and sex of the vital fetuses and length of their implantation sites was determined; implantation sites of non-viable/degenerated embryos were only counted. Placental length (between necrotic tips) was measured and placental weight was determined after drying (24h at 70 °C, followed by 4h at 103 °C). Embryonic survival was calculated as the total number of implantation sites divided by the number of corpora lutea, whereas fetal survival was calculated as the number of vital fetuses divided by the number of corpora lutea.

Blood sampling

Before weaning (at day - 0.5; 0730h feeding), and at day 2 (1530h feeding; batch 2 and 3) and day 2.5 (0730h feeding) after weaning, blood samples were taken from the ear vein catheter at - 24, - 12, 0, 12, 24, 36, 48, 60, 84, 120, 156, 228, 300, 372 and 444 min relative to feeding to assess glucose and insulin profiles. Additionally, at day - 1 (pre-weaning), 0, 1, 2 and 3 relative to weaning, blood samples were taken at 1500h (before feeding) to determine IGF-1 concentrations. At 12 - 13 days (by jugular venipuncture) and at 42 - 43 days (at slaughter) after estimated time of ovulation, additional blood samples were taken (prior to feeding) for progesterone determination. Blood samples were collected in

polypropylene tubes with 100 µl EDTA (Titriplex III, Merck Nederland B.V., Amsterdam, The Netherlands) solution (0.39M EDTA in saline), immediately placed on ice, centrifuged at 1710 x g for 10 min at 4 °C, and plasma was stored at - 20 °C until analyses.

Plasma analyses

Glucose and insulin

For glucose analyses, 500 µL 0.3M Trichloroacetic Acid (TCA) was added to 50 µL of plasma for precipitation of protein. After centrifugation at 16000 x g for 1 min, glucose concentrations in the supernatant were analyzed in triplicate with an enzymatic colorimetric assay using the glucose-oxidase-peroxidase (GOD-PAP) method using a commercial kit® (Roche Diagnostics Nederland BV, Almere, The Netherlands). Plasma insulin concentrations were analyzed in duplicate with a commercial RIA-kit (PI-12K Porcine Insulin RIA-kit®; Millipore, St. Charles, MO, USA). Sensitivity was 2 µU/ml, intra-assay CV was 6.4% (n = 42) and inter-assay CV was 6.0% (n = 9). For each sampling day, basal glucose and basal insulin concentrations were calculated as the mean value of the three samples taken before feeding (- 24, - 12 and 0 min); maximal insulin concentrations were defined as the maximum value during the first 156 min after feeding; and the area under the curve (AUC) was calculated as the area above basal glucose and insulin concentrations from feeding until 444 min after feeding.

Insulin-like growth factor-1

Plasma IGF-1 concentrations were quantified in duplicate, using a commercial kit (IRMA IGF-1 A15729®; Immunotech, Marseille, France), after extraction of the samples with ethanol/HCl [as validated by Louveau and Bonneau (1996)]. Sensitivity was 2 ng/ml, intra-assay CV was 2.2% (n = 26) and inter-assay CV was 3.5% (n = 12).

Progesterone

Progesterone concentrations were determined in duplicate, using a commercial Coat-A-Count Progesterone RIA-kit (PITKPG-7®; Siemens Medical Solutions Diagnostics, Los Angeles, CA, USA). Sensitivity was 0.1 ng/ml, intra-assay CV was 4.7% (n = 78) and inter-assay CV was 3.2% (n = 21).

Feed analyses

Diets were analyzed for dry matter (ISO 6496, 1999), crude fat (ISO 6492, 1999), Kjeldahl nitrogen (ISO 5983, 2005), starch (ISO 15914, 2004) and reducing sugars [as described by Van Vuuren et al. (1993)]. Crude protein was calculated as N x 6.25.

Statistical analyses

Three sows (two CON; one INS-H) had a low dry matter intake (DMI < 30%) during WEI and were therefore excluded from all analyses. The remaining 54 sows had a DMI ≥ 83% during WEI (until onset of estrus) and a 100% DMI at blood sampling days for glucose and insulin profiles. Due to lack of catheter patency, for one CON sow no glucose and insulin profiles were available before weaning.

Results are presented as LSmeans ± SEM, unless otherwise stated. Analysis of variance was applied to continuous data using the GLM procedure of SAS 9.1 (SAS Inst. Inc., Cary, NC, USA). The factors and interactions included in the statistical model were tested for significance and stepwise omitted from the model if $P > 0.05$ (except for the factor treatment). Bonferroni corrections were used for multiple comparisons.

To test whether glucose and insulin parameters and plasma IGF-1 concentrations differed between sampling days, the statistical model included treatment (CON, INS-L, INS-H), parity class (≤ 5 (n = 26), ≥ 6 (n = 28) at slaughter), batch (1, 2, 3), sow nested within treatment, parity class and batch, sampling day (2, 2.5 for glucose and insulin; 1, 2, 3 for IGF-1), and the interaction between treatment and sampling day. Additional analyses were done for each sampling day separately. To analyze whether insulin parameters at day 2 and day 2.5 were related to pre-weaning insulin parameters, pre-weaning insulin parameters and their interactions with treatment were added to the model.

Insulin concentrations were natural logarithm-transformed, because assumptions of normality were not fulfilled. For glucose and insulin profiles, significant interactions existed between treatment and sampling time and the variance of error terms was not uniform over time (variance decreases with time after feeding). Therefore, differences between treatments were analyzed for each sampling time separately (at day 2 and at day 2.5), with a statistical model that included treatment (CON, INS-L, INS-H), parity class (≤ 5 (n = 26), ≥ 6 (n = 28) at slaughter), and batch (1, 2, 3).

To evaluate treatment effects on reproduction characteristics, the statistical model included treatment (CON, INS-L, INS-H), parity class (≤ 5, ≥ 6 at slaughter), batch (1, 2, 3) and the interactions between treatment and batch and between treatment and parity class. For luteal development and progesterone concentrations and for fetal and placental development, sampling time (12 or 13 days for progesterone) or slaughter time (42 or 43 days) was added as additional factor. For number of vital fetuses and implantation sites, additional analyses were done adding ovulation rate as covariate. For fetal and placental development, additional analyses were done adding number of implantation sites as a covariate. To check whether lactation characteristics (insulin parameters before weaning, IGF-1 concentrations before weaning, body weight loss (in %) and backfat loss (in mm) during lactation) interacted with the effect of treatment, these characteristics and their interaction with treatment were added to the models.

The percentage of sows showing estrus ≤ 7 days and pregnancy rate at day 42 - 43 were analyzed using the LOGISTIC procedure. The model included treatment (CON, INS-L, INS-H), parity class (≤ 5, ≥ 6 at slaughter), batch (1,2,3) and the interactions between treatment and batch and between treatment and parity class.

Pearson correlations were assessed among insulin parameters, IGF-1 concentrations and sow body condition characteristics. To study relations between insulin parameters (before weaning and at day 2.5), IGF-1 concentrations (before weaning and at day 3) or sow body condition characteristics (sow body weight and backfat thickness at weaning, sow body weight and backfat loss during lactation) and reproduction characteristics, the statistical model contained an insulin, IGF-1 or sow body condition characteristic as factor (divided into three classes: 25% lowest, 50% average, and 25% highest observations). When reproduction characteristics were significantly affected by treatment (except for insulin parameters at day 2.5), batch, sampling/slaughter time, ovulation rate or number of implantation sites, these factors or covariates were added to the model. When effects of insulin, IGF-1 or sow body condition characteristics seemed linear, additional analyses were done with the insulin, IGF-1 or sow body condition characteristics analyzed as covariate instead of class variable, using the same model.

RESULTS

Effect of treatment

Average DMI during WEI (until onset of estrus) was 98.9 ± 0.4%.

Glucose

Glucose profiles are shown in Figure 5.1. Glucose parameters differed between day 2 (1530h) and day 2.5 (0730h) for all treatments; basal glucose concentration was higher at day 2 than at day 2.5 (80.2 mg/dl at day 2 and 71.7 mg/dl at day 2.5, SEM = 1.2, $P < 0.001$), whereas glucose AUC was lower at day 2 than at day 2.5 (1351 mg/444 min at day 2 and

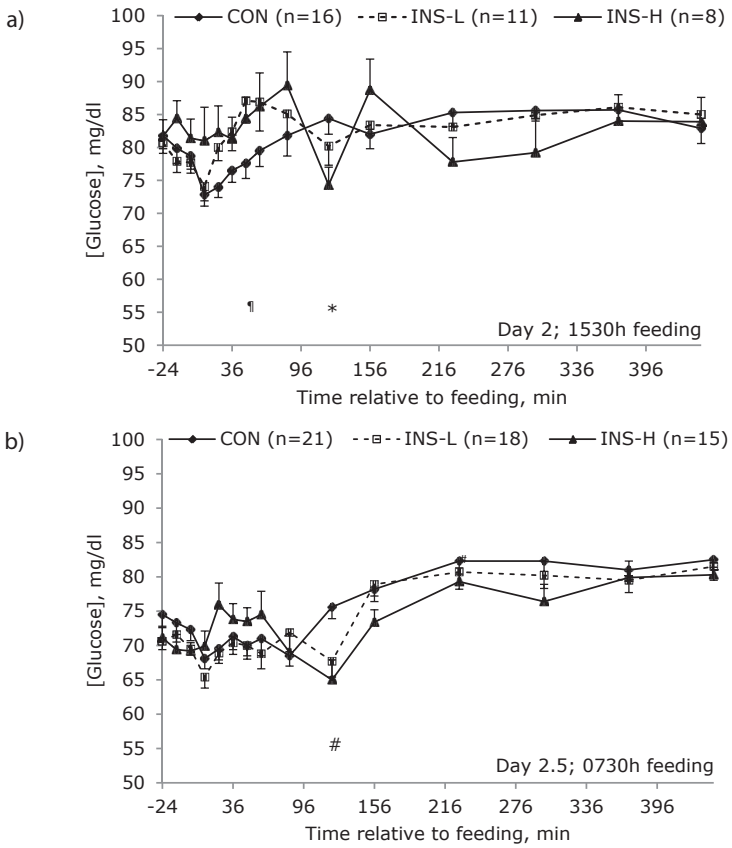

Figure 5.1 Glucose profiles at **a)** day 2 (1530h feeding) and **b)** day 2.5 (0730h feeding) after weaning for sows fed a diet supplemented with either 375 g/day dextrose plus 375 g/day starch (INS-H) or with 172 g/day dextrose plus 172 g/day starch (INS-L), or a control diet (CON) during WEI (means ± SE). * = CON vs. INS-H, $P \leq 0.05$; # = CON vs. INS-L and INS-H, $P \leq 0.05$; ¶ CON vs. INS-L, $P \leq 0.05$.

2751 mg/444 min at day 2.5, SEM = 449, $P = 0.03$). Glucose parameters were not affected by treatment (Table 5.2).

Insulin

Insulin AUC was lower at day 2 (1530h) than at day 2.5 [0730h; 5892 µU/444 min at day 2 and 7478 µU/444 min at day 2.5, SEM = 379, $P < 0.01$] for all treatments, but the interaction between treatment and sampling day for maximal insulin ($P < 0.01$) indicated that maximal insulin was significantly lower at day 2 than at day 2.5 for INS-H only (85.6 µU/ml at day 2 and 137.3 µU/ml at day 2.5, SEM = 5.5). Therefore, treatment effects on insulin profiles (Figure 5.2) and insulin parameters (Table 5.2) are analyzed for each sampling day separately.

Table 5.2 Glucose and insulin parameters and IGF-1 concentrations during WEI (weaning is day 0) for sows fed a diet supplemented with either 375 g/day dextrose plus 375 g/day starch (INS-H) or with 172 g/day dextrose plus 172 g/day starch (INS-L), or a control diet (CON) (LSmeans ± SEM)

	Treatment				
Item	CON	INS-L	INS-H	SEM	*P*-value
Glucose and insulin					
Day 2 (1530h)					
Number of sows	16	11	8		
Basal glucose, mg/dl	80.1	79.1	82.5	1.9	0.49
Glucose AUC, mg/444 min	1329	2232	- 121	831	0.19
Basal insulin, µU/ml	14.5	17.7	12.9	1.4	0.08
Maximal insulin, µU/ml	56.5	82.5	85.6	9.6	0.05
Insulin AUC, µU/444 min	4120[a]	5749[ab]	7331[b]	633	< 0.01
Day 2.5 (0730h)					
Number of sows	21	18	15		
Basal glucose, mg/dl	73.4	70.6	69.9	1.4	0.16
Glucose AUC, mg/444 min	2071	2829	2590	528	0.56
Basal insulin, µU/ml	15.7	16.3	16.3	1.2	0.90
Maximal insulin, µU/ml	70.2[a]	116.6[b]	150.5[b]	10.3	< 0.001[1]
Insulin AUC, µU/444 min	4516[a]	8197[b]	10894[c]	694	< 0.001
IGF-1					
Number of sows	21	18	15		
Day 1, ng/ml	108	115	108	8.6	0.81
Day 2, ng/ml	132	140	139	7.9	0.69
Day 3, ng/ml	149	159	166	6.9	0.23

[1] Significantly affected by parity ($P = 0.02$); LS means were 126.3 µU/ml for parity ≤ 5 and 98.5 µU/ml for parity ≥ 6.
[abc] Within treatment, values lacking a common superscript differ ($P \leq 0.05$).

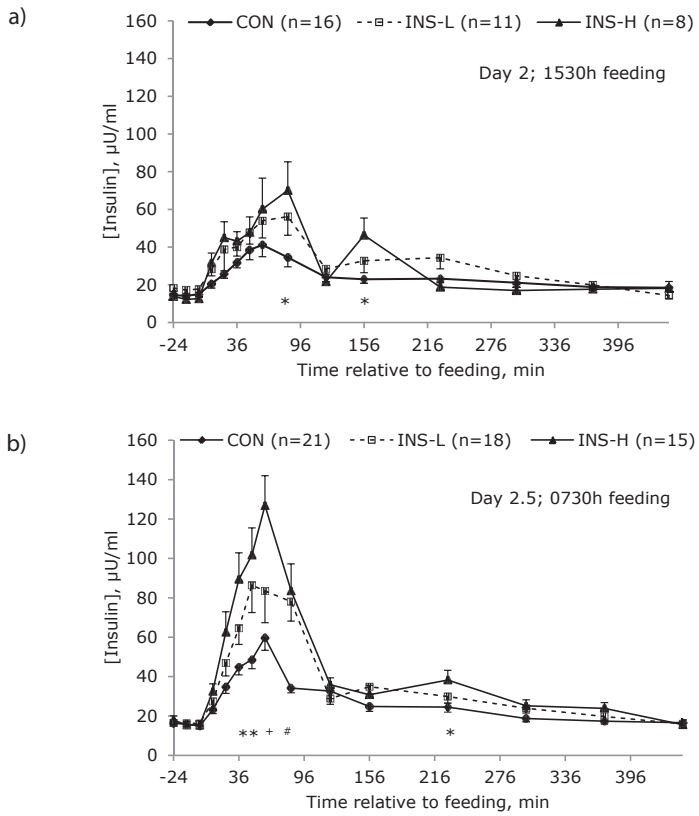

Figure 5.2 Insulin profiles at **a)** day 2 (1530h feeding) and **b)** day 2.5 (0730h feeding) after weaning for sows fed a diet supplemented with either 375 g/day dextrose plus 375 g/day starch (INS-H) or with 172 g/day dextrose plus 172 g/day starch (INS-L), or a control diet (CON) during WEI (means ± SE). * = CON vs. INS-H, $P \leq 0.05$; $^{+}$ = INS-H vs. CON and INS-L, $P \leq 0.05$; $^{\#}$ = CON vs. INS-L and INS-H, $P \leq 0.05$.

5

At day 2 (1530h feeding), insulin AUC was higher for INS-H than for CON (Δ = 3211 µU/444 min; $P < 0.01$; Table 5.2). At day 2.5 (0730h feeding), maximal insulin concentrations were higher for INS-L and INS-H than for CON (Δ = 46 and 80 µU/ml, respectively; $P < 0.001$), and insulin AUC differed between all three treatments ($P < 0.001$; Table 5.2).

Insulin-like growth factor-1

Plasma IGF-1 concentrations increased during WEI (111 ng/ml at day 1, 137 ng/ml at day 2 and 158 ng/ml at day 3; SEM = 2.0; $P < 0.001$), but IGF-1 concentrations up to 3 days after weaning were not affected by treatment (Table 5.2).

Reproduction characteristics

Weaning-to-estrus interval and follicle development were not affected by treatment, but estrus duration was shorter in INS-H than in CON (Δ = 12h, $P = 0.03$; Table 5.3). Pregnancy rate at day 42 - 43 (Table 5.4), luteal development (Table 5.3), and embryonic and fetal survival (Table 5.4) were not affected by treatment. Mean fetal length was higher for INS-L than for INS-H (Δ = 2.1 mm, $P = 0.04$), whereas placental dry weight CV was lower for INS-L than for INS-H (Δ = 5.5%, $P = 0.03$; Table 5.4).

Effects of previous lactation characteristics

During previous lactation, sows lost on average 22.9 ± 1.6 kg (8.8 ± 0.6%) of body weight and 4.6 ± 0.3 mm of backfat. Before weaning (at day - 0.5; 0730h), basal glucose concentration

Table 5.3 Follicle development, estrus and luteal development characteristics of sows fed a diet supplemented with either 375 g/day dextrose plus 375 g/day starch (INS-H) or with 172 g/day dextrose plus 172 g/day starch (INS-L), or a control diet (CON) during WEI (LSmeans ± SEM)

	Treatment				
Item	CON	INS-L	INS-H	SEM	*P*-value
Follicle development and estrus					
% sows showing estrus ≤ 7 days	90% (19/21)[1]	94% (17/18)	100% (15/15)		0.70
WEI[2], h	97	103	107	5	0.40
Estrus duration[2], h	64[b]	64[ab]	52[a]	3	0.03
Follicle diameter at day 0.5[2], mm	4.1	3.9	3.9	0.1	0.61
Follicle diameter at day 4.5[2,3], mm	6.4	6.3	6.5	0.2	0.78
Luteal development					
Number of sows[4]	16	15	15		
Ovulation rate	26.9	25.5	28.8	1.1	0.13
Total luteal weight, g	9.4	9.2	9.7	0.5	0.76
Mean corpus luteum weight, g	0.34	0.35	0.35	0.02	0.87[5]
SD, g	0.06	0.06	0.05	0.01	0.67
CV, %	17.7	16.4	14.5	1.9	0.49
Progesterone at day 12 - 13, ng/ml	40.8	36.8	37.7	1.7	0.22[6]
Progesterone at day 42 - 43, ng/ml	26.1	27.9	29.9	2.0	0.41

[1] Of which one sow ovulated (this sow is included in analyses on follicle diameter).
[2] Of sows with WEI ≤ 7 days.
[3] Excluding three CON sows that had already ovulated at day 4.5.
[4] Excluding sows that were not pregnant (incl. one CON sow that aborted on day 27 of pregnancy), except that for ovulation rate and progesterone at day 12 non-pregnant sows were included.
[5] After correction for ovulation rate ($P < 0.001$), *P*-value for treatment was 0.74.
[6] Corrected for sampling moment ($P = 0.01$).
[ab] Within treatment, values lacking a common superscript differ ($P \leq 0.05$).

was 65.1 ± 1.1 mg/dl, glucose AUC was 8494 ± 542 mg/444 min, basal insulin concentration was 8.0 ± 0.8 μU/ml, maximal insulin concentration was 135.6 ± 9.1 μU/ml and insulin AUC was 11228 ± 786 μU/444 min. Plasma IGF-1 concentrations were 134 ± 7 ng/ml before weaning (at day - 1) and 119 ± 6 ng/ml at weaning (day 0). No differences existed in these lactation characteristics among treatments. The interactions of these previous lactation characteristics with treatment were never significant, indicating that treatment

Table 5.4 Fetal and placental development characteristics of sows fed a diet supplemented with either 375 g/day dextrose plus 375 g/day starch (INS-H) or with 172 g/day dextrose plus 172 g/day starch (INS-L), or a control diet (CON) during WEI (LSmeans ± SEM)

	Treatment				
Item	CON	INS-L	INS-H	SEM	*P*-value
Pregnancy rate at day 42 - 43[1]	84% (16/19)	88% (15/17)	100% (15/15)		0.33
Fetal development					
Implantation sites, n	21.1	21.7	23.4	1.1	0.35[2]
Embryonic survival, %	76	83	82	4	0.39
Vital fetuses, n	18.0	18.5	19.3	1.1	0.69
Fetal survival, %	65	71	68	4	0.59
Sexratio, prop. males	0.51	0.48	0.53	0.02	0.27
Mean fetal weight, g	16.5	17.3	16.3	0.5	0.23[3]
SD, g	1.7	1.6	1.7	0.1	0.80[3]
CV, %	10.4	9.5	10.6	0.6	0.39
Mean fetal length, mm	66.5[ab]	68.1[b]	66.0[a]	0.7	0.04[3]
SD, mm	2.6	2.6	2.8	0.2	0.70
CV, %	3.9	3.9	4.3	0.3	0.51
Placental development					
Mean placental length, cm	41.9	44.0	40.8	1.5	0.32[4]
SD, cm	11.4	10.6	11.6	0.6	0.42
CV, %	27.2	24.4	29.0	1.3	0.06[4]
Mean placental dry weight, g	3.5	3.8	3.5	0.1	0.20[4]
SD, g	0.9	0.9	1.0	0.1	0.33
CV, %	26.9[ab]	22.9[a]	28.4[b]	1.5	0.03[4]
Mean implantation length, cm	20.5	21.6	20.4	1.1	0.71
SD, cm	6.0	6.1	6.5	0.4	0.54
CV, %	29.2	28.5	32.0	1.4	0.18

[1] Of sows with WEI ≤ 7 days.
[2] After correction for ovulation rate ($P = 0.02$), *P*-value for treatment was 0.39.
[3] Corrected for slaughter time ($P < 0.05$).
[4] After correction for number of implantation sites ($P < 0.05$), *P*-values were 0.34 for mean placental length, 0.09 for placental length CV, 0.16 for mean placental dry weight, and 0.04 for placental dry weight CV.
[ab] Within treatment, values lacking a common superscript differ ($P \leq 0.05$).

effects on reproduction characteristics were never affected by these previous lactation characteristics (data not shown).

Relationships between insulin, IGF-1 and reproduction characteristics

Relationships among insulin and IGF-1

Pre-weaning insulin parameters were not related to insulin parameters at day 2 or day 2.5 (after corrections for treatment), but IGF-1 concentrations at all sampling days were highly correlated ($r \geq 0.63$, $P < 0.01$). Pre-weaning insulin response parameters (maximal and AUC) were positively correlated to IGF-1 concentrations before weaning and at day 0 and 1, whereas insulin response parameters at day 2.5 were positively correlated to IGF-1 concentrations at day 2 and 3 (Table 5.5).

Relationships between insulin or IGF-1 and reproduction characteristics

Pre-weaning plasma IGF-1 concentration was negatively related to WEI (WEI (h) = 122.92 – 0.16 x pre-weaning IGF-1 concentration (ng/ml); r = - 0.37; $P < 0.01$). Maximal insulin concentration at day 2.5 was positively related to progesterone at day 42 - 43 (progesterone concentration at day 42 - 43 (ng/ml) = 22.50 + 0.05 x maximal insulin concentration at day 2.5 (µU/ml); $r = 0.35$; $P = 0.02$).

Table 5.5 Pearson correlations between insulin parameters and IGF-1 concentrations[1]

	IGF-1 concentration, ng/ml				
Insulin parameter	Day -1	Day 0[2]	Day 1	Day 2	Day 3
Pre-weaning insulin					
(Day - 0.5; n = 53)					
Basal, µU/ml	n.s.[3]	n.s.	n.s.	n.s.	n.s.
Maximal, µU/ml	0.44**	0.45**	0.37**	n.s.	n.s.
AUC, µU/444 min	0.39**	0.42**	0.32*	n.s.	n.s.
Insulin day 2.5					
(n = 54)[4]					
Basal, µU/ml	n.s.	n.s.	n.s.	n.s.	n.s.
Maximal, µU/ml	n.s.	n.s.	n.s.	0.31*	0.44***
AUC, µU/444 min	n.s.	n.s.	n.s.	0.29*	0.37**

[1] Insulin parameters at day 2 (n = 35) were not correlated with IGF-1 concentrations.
[2] Day 0 = day of weaning.
[3] n.s. = not significant ($P > 0.05$).
[4] For insulin parameters at day 2.5, relationships corrected for treatment gave similar results (the interaction between treatment and insulin parameter was never significant).
* $P \leq 0.05$; ** $P \leq 0.01$; *** $P \leq 0.001$.

Relationships between sow body condition and reproduction characteristics

Relationships among insulin, IGF-1 and sow body condition

Sow body weight at weaning was positively correlated to IGF-1 concentrations before weaning ($r = 0.33$, $P = 0.02$) and at weaning ($r = 0.39$, $P < 0.01$), and sow backfat thickness at weaning was positively correlated to IGF-1 concentrations before weaning ($r = 0.42$, $P < 0.01$) and at weaning ($r = 0.49$, $P < 0.01$) and to maximal insulin before weaning ($r = 0.30$, $P = 0.03$). Body weight loss during lactation was negatively correlated to IGF-1 concentrations before weaning ($r = -0.42$, $P < 0.01$ for both kg and %) and at weaning ($r = -0.43$, $P < 0.01$ for both kg and %), and to maximal insulin ($r = -0.38$, $P < 0.01$ for both kg and %) and insulin AUC ($r = -0.32$, $P = 0.02$ for both kg and %) before weaning.

Relationships between sow body condition and reproduction characteristics

Sow backfat loss during lactation was positively related to mean implantation length (mean implantation length (cm) = 17.25 + 0.77 x backfat loss during lactation (mm); $r = 0.38$; $P < 0.01$). No other clear relationships were found.

DISCUSSION

This study was designed to evaluate effects of different levels of insulin-stimulating feed components (dextrose plus starch; fed twice daily) during WEI, on accompanying plasma insulin and IGF-1 concentrations, on follicle development and on subsequent luteal, fetal and placental development and uniformity in multiparous sows. The dextrose plus starch-diets effectively enhanced the postprandial insulin response in a dose-dependent manner, but did not affect plasma IGF-1 concentrations in the first 3 days after weaning. Follicle development and subsequent luteal, fetal and placental development were not affected by the dietary treatments, nor related to plasma insulin and IGF-1 concentrations during the first 3 days of WEI.

Recently, we found that sows that lost more than 13% of their body weight or more than 5 mm of their backfat during previous lactation had a lower subsequent litter uniformity compared to sows that lost less than 3.5% of their body weight or less than 2 mm of their backfat (Wientjes et al. in press). This indicates that severe body condition loss during previous lactation is a risk factor for low litter uniformity, which may be related

to suppressed insulin levels and suppressed follicle development. Insulin-stimulating sow diets before mating can improve subsequent litter uniformity (Van den Brand et al. 2006, 2009). In a previous study, in which sows lost 12.0 ± 0.5% of their body weight during lactation, plasma insulin concentrations during WEI were positively related to LH, follicle development and subsequent luteal and embryo development at day 10 of pregnancy (Wientjes et al. 2012b, c). In the current study, sows lost only 8.8 ± 0.6% of their body weight during lactation, and pre-mating insulin profiles were not related to follicle development or subsequent development and uniformity of fetuses and placentas. Insulin-stimulating diets during WEI may, therefore, only be beneficial for follicle development and subsequent litter uniformity in sows with suboptimal follicle development at weaning, e.g. due to severe body condition loss. In the current study, sows had follicles of 4.0 ± 0.1 mm at weaning and 27.0 ± 0.6 subsequent ovulations, which may indicate that follicle development was not suppressed in these high prolific sows.

The postprandial insulin response, both insulin peak concentrations (within an hour after feeding) and long-term insulin concentrations (around 4h after feeding), increased with the dextrose and starch content of the diet, likely reflecting the separate effects of dextrose (rapid available glucose) and starch (slower available glucose), respectively. The contrast in postprandial insulin response between the INS-L diet (172 g/day dextrose plus 172 g/day corn starch) and the control diet in this study was considerably higher than the contrast found in a previous study comparing a dextrose plus lactose-diet (both 150 g/day) with a control diet [+ 67% vs. + 50% in peak concentration, + 82% vs. + 34% in AUC; Wientjes et al. (2012a)] in sows. In gilts, Van den Brand et al. (1998) found a higher insulin response (+ 14% in peak concentration, + 44% in AUC) for a diet supplemented with dextrose plus corn starch (60 g/kg dextrose; 178 g/kg corn starch) compared to a diet supplemented with only corn starch (238 g/kg corn starch). Dietary supplementation with dextrose plus starch, thus, seems to be more effective in stimulating insulin secretion than supplementation with only starch or dextrose plus lactose. The comparable plasma glucose responses for the three diets in the present study indicate that these sows were highly able to cope with differences in glucose availability among the diets by secretion of sufficient insulin.

The postprandial insulin response was higher after morning feeding than after afternoon feeding, as observed earlier in sows (Valros et al. 2003), growing pigs (Malmlöf et al. 1990), and human (Jarrett et al. 1972), using equal feed portions at both feedings. This may be related to the longer fasting period before the morning feeding (16h)

compared to the afternoon feeding (8h). In pigs, after 12 - 20h of fasting, plasma glucose concentrations start to gradually decrease and plasma free fatty acid concentrations (FFA) start to gradually increase (Barb et al. 1997; Inoue et al. 2005). Indeed in our sows, pre-prandial glucose concentrations were lower in the morning than in the afternoon, and it is likely that corresponding pre-prandial plasma FFA concentrations were higher in the morning than in the afternoon. Short-term increases in plasma FFA are reported to stimulate glucose-stimulated insulin secretion in rats and humans (Haber et al. 2003; Itoh et al. 2003). On the other hand, comparable diurnal variation in insulin responses was observed in studies with identical meals at identical time intervals, in growing pigs (Koopmans et al. 2005), humans (La Fleur 2003), and rats (Kalsbeek and Strubbe 1998), suggesting the existence of a circadian rhythm in insulin responses, independent of time intervals between feedings. Koopmans et al. (2005) suggested that insulin responses are higher in the morning to counteract the catabolic pressure of cortisol, which also peaks in the morning. In human, a decrease in pancreatic β-cell responsiveness to glucose and/or a decrease in insulin sensitivity during the day are reported to result in lower glucose tolerance in the afternoon than in the morning (e.g. Service et al. 1983; La Fleur 2003). Thus, the higher postprandial insulin responses in the morning compared to the afternoon, as observed in our study, were probably mediated by several mechanisms. Because insulin responses differ between morning and afternoon feedings, studies investigating effects of insulin on e.g. follicle development should consider both insulin responses.

It is well known that during a period of insulin deficiency, e.g. due to a catabolic state during lactation, hepatic growth hormone (GH) binding, and thereby GH-stimulated IGF-1 production, is inhibited (Clemmons and Underwood 1991; Thissen et al. 1994). Indeed in our sows, insulin and IGF-1 concentrations before weaning were negatively related to sow body weight loss during lactation, and positively related to sow backfat thickness at weaning, as also observed by Hoving et al. (2012) and Rojkittikhun et al. (1993). After weaning, sows change towards an anabolic state, associated with restoration of plasma insulin and IGF-1 concentrations (Van den Brand et al. 2001; Wientjes et al. 2012b). In our sows, after weaning a quick restoration of insulin secretion took place and no relations existed between pre- and post-weaning insulin levels. Restoration of IGF-1 secretion, however, took longer and plasma IGF-1 concentrations during the first 3 days of WEI were not affected by the insulin-stimulating diets. This may indicate that modulation of plasma IGF-1 concentrations by insulin-stimulating diets after weaning is limited, as has also

been found previously in anabolic sows (Wientjes et al. 2012a). However, positive relations between IGF-1 concentrations and insulin parameters were stronger at day 3 after weaning than at day 2, and plasma IGF-1 concentrations started to diverge between the diets on day 3 after weaning. This may indicate that insulin-stimulating diets during WEI result in higher plasma IGF-1 levels from 4-5 days after weaning onwards. Due to this possible latency in IGF-1 response to insulin-stimulating diets after weaning, however, the WEI may be too short for an effective stimulation of IGF-1 secretion by insulin-stimulating diets. Further, plasma IGF-1 concentrations during the first days of WEI were strongly related to pre-weaning IGF-1 concentrations. To stimulate IGF-1 concentrations during (the first days of) WEI, and thereby possibly influence follicle development, focus should be on increased IGF-1 concentrations during late lactation. In primiparous sows, both an increased feeding level and insulin-stimulating diets were effective stimulators of plasma IGF-1 concentrations during lactation (Van den Brand et al. 2001).

To conclude, this study shows that dietary dextrose plus starch effectively stimulate insulin secretion (both postprandial peak and long term concentration), but not IGF-1 secretion during the first 3 days after weaning in multiparous sows. Extreme insulin-stimulating diets during WEI, however, did not improve development of follicles, and subsequent development and uniformity of fetuses and placentas in our high prolific sows. Insulin-stimulating diets during WEI may, therefore, only be beneficial for follicle development and subsequent litter uniformity in sows with suboptimal follicle development at weaning.

IMPLICATIONS

In European pig husbandry, one out of five piglets dies before weaning, which is a major economic and welfare problem. For piglet survival, and for piglet performance before and after weaning, high piglet birth weights and litter uniformity are crucial. A way to improve piglet birth weights and litter uniformity could be the use of insulin-stimulating sow diets before mating, probably through beneficial effects on follicle development. Our results implicate that in weaned high prolific sows, dextrose plus starch are effective insulin-stimulating feed components, but may not improve piglet birth weight and litter uniformity.

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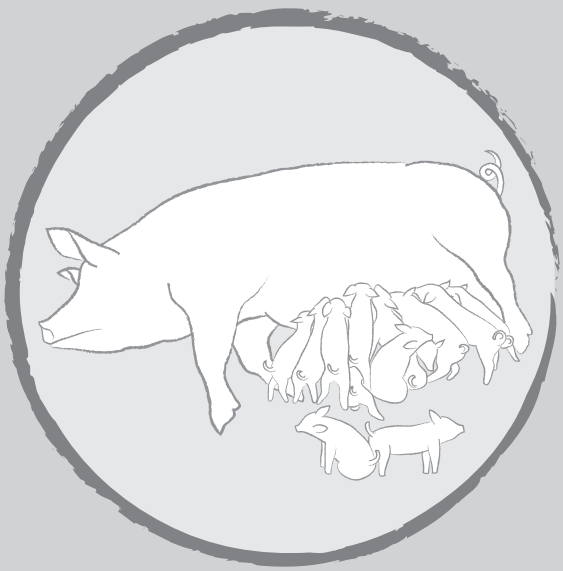
Chapter 6

Piglet uniformity and mortality in large organic litters: Effects of parity and pre-mating diet composition

JGM Wientjes[1]
NM Soede[1]
CMC van der Peet-Schwering[2]
H van den Brand[1]
B Kemp[1]

[1] Adaptation Physiology Group, Department of Animal Sciences, Wageningen University, Wageningen, The Netherlands

[2] Wageningen UR Livestock Research, Lelystad, The Netherlands



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ABSTRACT

In large organic litters, relationships between piglet birth weight, piglet uniformity and pre-weaning piglet mortality were studied. Furthermore, effects of parity and insulin-stimulating diets during the pre-mating period on piglet birth weight, uniformity, and mortality were investigated. Organically kept sows (n = 137 sow cycles) were fed a control diet during lactation and weaning-to-insemination interval (CON), or an insulin-stimulating diet (sucrose plus lactose, both 150 g/day) during only the weaning-to-insemination interval (WII) or during the last two weeks of a 41 ± 4 day lactation and the weaning-to-insemination interval (LAC + WII). Piglets (live born and stillborn) were weighed individually within 24h after birth. Cross-fostering was allowed within treatments within the first 3 days after birth. Litter size was higher for parity 3 and 4 sows than for older sows, whereas parity 2 sows had an intermediate litter size (the number of total born piglets was 17.0, 18.8 and 16.3 for sows of parity 2, 3 + 4 and ≥ 5, respectively; $P < 0.01$). Mean birth weight (1.26 ± 0.02 kg) was not influenced by parity, but birth weight CV and percentage of piglets < 800 g increased with increasing parity class, after corrections for number of total born piglets (for parity 2, 3 + 4 and ≥ 5, respectively, CV of birth weights were 21.3, 23.2 and 24.8%, $P = 0.05$; and % piglets < 800 g were 6.2, 8.7 and 13.6%, $P = 0.02$). Pre-weaning piglet mortality also increased with parity class (20.9, 24.2, and 33.3% for parity 2, 3 + 4 and ≥ 5, respectively; $P = 0.01$). Litter characteristics at birth and pre-weaning piglet mortality were not affected by the insulin-stimulating diets before mating. Piglet mortality from days 0 to 3 was strongly related to the number of total born piglets ($\beta = 1.47$ %/piglet; $P < 0.001$), mean birth weight of the piglets ($\beta = -30.99$ %/kg; $P < 0.001$), CV of birth weights ($\beta = 1.08$ %/%; $P < 0.001$) and % piglets < 800 g ($\beta = 0.58$ %/%; $P < 0.01$). It is concluded that piglet birth weight and birth weight uniformity affect pre-weaning piglet mortality in organic sows with large litters. Piglet uniformity and piglet mortality were also affected by parity, but not by pre-mating insulin-stimulating diets.

INTRODUCTION

Dutch organic sows farrow larger litters than conventional sows (Leenhouwers et al. 2011). However, organic sows wean fewer piglets per litter than conventional sows, due to a high pre-weaning piglet mortality during their six-week lactation period (Leenhouwers et al. 2011).

Two important factors affecting pre-weaning piglet mortality are piglet birth weight and piglet uniformity (Milligan et al. 2002; Quiniou et al. 2002). Both piglet birth weight and uniformity are negatively related to litter size (Milligan et al. 2002; Quesnel et al. 2008), at least in sow populations with average litter sizes up to 13 - 14 total born piglets. Piglet birth weight and uniformity are also affected by parity (Milligan et al. 2002; Damgaard et al. 2003; Quesnel et al. 2008), as is pre-weaning piglet mortality (Marchant et al. 2000; Milligan et al. 2002; Weber et al. 2007, 2009), but it is not clear whether this is related to differences in litter sizes among parities.

A way to improve piglet birth weight and litter uniformity could be the use of insulin-stimulating sow diets (dextrose plus lactose) before mating (Van den Brand et al. 2006, 2009), probably through beneficial effects of insulin on follicle development [as reviewed by e.g. Poretsky and Kalin (1987); Quesnel (2009)] and subsequent embryo and luteal development (Wientjes et al. 2012b, c). These studies have been performed in conventional sows with lactation lengths of 24 to 28 days, which have a strong catabolic state until weaning, associated with suppressed insulin levels and suppressed follicle development [as reviewed by e.g. Quesnel (2009)]. The question arises whether insulin-stimulating diets during the pre-mating period can also improve the low piglet birth weight and piglet uniformity in organic sows, with much longer lactation lengths (approx. 40 days), which may affect sow metabolic state until weaning.

Therefore, the aim of this experiment was to study relationships between piglet birth weight, uniformity and pre-weaning mortality in organic sows with large litters and of different parities, and to investigate whether insulin-stimulating diets during different stages of the pre-mating period can improve piglet birth weight and uniformity, and thereby reduce piglet mortality, of these large litters.

MATERIALS AND METHODS

General design

Organic sows were followed from entering the farrowing stable until weaning of the subsequent litter, and were fed a control diet during lactation and weaning-to-insemination interval, or an insulin-stimulating diet during the weaning-to-insemination interval or during the last two weeks of lactation and the weaning-to-insemination interval. Care and treatment of animals was according to Dutch animal welfare legislation.

Animals and housing

At Pig Research Centre Raalte of Wageningen University and Research Centre, in total 137 sow cycles of 89 organically kept Topigs 20 sows (Topigs, Vught, The Netherlands) were studied in 14 consecutive batches of 9 - 15 sows.

From 14 ± 6 days before farrowing, sows were kept individually in indoor farrowing pens, which consisted of a solid floor bedded with straw (2.0 x 2.25 m) and a slatted floor (2.0 x 1.5 m), with access to an outdoor pen (2.0 x 1.75 m). Sows were exposed to 11h of artificial light per day (0700 - 1800h). The farrowing pens contained a piglet area, separated from the sow, also bedded with straw. The solid floor and piglet area contained floor heating; in the piglet area floor heating was on from expected farrowing until weaning (gradually declining from 35 °C to 19 °C), in the lying area of the sow floor heating only started when room temperature dropped below 16 °C. To standardize litter sizes, cross-fostering of piglets was allowed within treatments within the first 3 days after birth. Weighing of piglets (within 24h after birth) was combined with iron injections and ear tagging. Within 7 days after birth male piglets were castrated under general anaesthesia.

After weaning (at 41 ± 4 days) and during pregnancy, sows were group housed (in stable groups of 9 - 15 sows) in pens that were partly bedded with straw, and with access to an outdoor pen. Sows were fixated in stalls during feeding and during estrus. From April to October, pregnant sows had free access to pasture. Sows were exposed to 24h of artificial light per day from weaning until 3 days after weaning, and 11h of artificial light per day (0700 - 1800h) thereafter.

From 3 days after weaning, sows were checked for estrus twice daily (at 0800h and 1330h) using a mature boar. All sows were inseminated each day of estrus with a dose of

commercially available semen (containing 2 x 10^9 sperm cells) of a Pietrain boar line. At 18 - 22 days after insemination sows were checked for estrus using a mature boar. At 28 days after insemination sows were checked for pregnancy using transcutaneous ultrasonography.

Feeding and dietary treatments

In the farrowing rooms, sows were fed twice daily (at 0730h and 1400h). From entering the farrowing room until day 109 of pregnancy sows received a standard organic pregnancy feed (12.2 MJ ME/kg; 151 g/kg CP; 6.8 g/kg lysine). From day 109 until day 113 of pregnancy, sows were gradually switched to a standard organic lactation feed (CON diet; Table 6.1).

From entering the farrowing room until farrowing, sows received 3.6 - 4.2 kg feed per day, depending on room temperature. On the day of farrowing, sows received 1.5 kg feed. During the first 6 days after farrowing, feed allowance was gradually increased to 6.5 kg per day. From day 14 of lactation until weaning, sows received 7.5 kg feed per day. Piglets were fed an organic creep feed from two weeks of age onwards.

Within each batch, sows were allocated to one of three dietary treatments, based on parity and sow body weight at entering the farrowing stable: CON: control diet during lactation and weaning-to-insemination interval; WII: sucrose plus lactose (both 150 g/day) during the weaning-to-insemination interval; or LAC + WII: sucrose plus lactose (both 150 g/day) during the last two weeks of lactation and weaning-to-insemination interval. Sucrose and lactose (both organic) were used as insulin-stimulating feed components. Sucrose was used as an alternative for dextrose, because no organic dextrose was available. Dextrose and sucrose do not differ in their insulin-stimulating effect in sows (Wientjes et al. 2012a).

During lactation, sows of the CON and WII treatments received the standard organic lactation feed (CON diet, Table 6.1). The LAC + WII sows received the standard organic lactation feed (CON diet, Table 6.1) until 2 weeks before weaning. Thereafter, they received an isocaloric lactation diet with 20 g/kg sucrose plus 20 g/kg lactose (SL diet; Table 6.1), resulting in a daily intake of approximately 150 g sucrose plus 150 g lactose at a feeding level of 7.5 kg.

From weaning until insemination, all sows were fed 3 kg of the standard organic lactation feed (CON diet; Table 6.1) once daily, and the WII and LAC + WII sows received a topdressing of 150 g sucrose plus 150 g lactose per day.

Table 6.1 Composition of the lactation diets (as fed)

	Lactation diet[1]	
Ingredient	CON, g	SL, g
Potato protein	32.2	36.6
Rapeseed expeller	40.0	13.8
Sugarcane molasses	34.5	35.0
Limestone	9.3	11.6
Monocalciumphosphate	10.1	10.4
Organic maize	401.9	294.5
Organic wheat	-	86.8
Organic rye	75.0	75.0
Organic wheat middlings	50.0	95.3
Organic sunflowerseed expeller	74.0	79.1
Organic peas	125.0	125.5
Organic alfalfa	75.0	42.5
Organic soybean expeller	22.6	43.0
Organic rapeseed expeller	40.0	-
Organic sucrose	-	20.0
Lactose	-	21.0
Salt	5.4	5.4
Vitamin - mineral premix	5.0	5.0
Total, g	**1000**	**1000**
Calculated content	g/1000 g	g/1000 g
Crude fat	42.0	34.6
Crude protein	168.2	162.5
Starch	358.8	350.0
Sugar	45.9	66.0
kJ NE (for swine)[2]	9064	9064
Digestible lysine[3]	6.6	6.6
Digestible methionine + cystine[3]	4.8	4.6

[1] Sows of the CON and WII treatments received the CON-diet until weaning. The LAC + WII sows received the CON diet until 2 weeks before weaning. Thereafter they received the SL (sucrose plus lactose) diet until weaning.
[2] According to the Centraal Veevoederbureau (CVB 2007).
[3] Apparent ileal digestibility (CVB 2007).

During subsequent pregnancy, sows received a standard organic pregnancy feed (12.2 MJ ME/kg; 151 g/kg CP; 6.8 g/kg lysine) once daily at 2.4 kg/day (until day 35), 2.6 kg/day (days 36 - 85) or 3.1 kg/day (day 86 until entering the farrowing stable).

All sows had daily access to organic straw as roughage throughout the experiment. Water was available *ad libitum* for sows and piglets during the whole experiment.

Measurements

Sows were weighed before entering the farrowing stables (at 14 ± 6 days before farrowing) and at weaning (at 41 ± 4 days). Piglets (live born and stillborn) were weighed individually within 24h after birth and at weaning. Sow body weight at farrowing was estimated by subtracting the weight of the litter at birth (live born and stillborn), estimated placenta and amnion weight [216 g/piglet; based on Van Rens and Van der Lende (2002)], and estimated fetal growth during the days between weighing of the sow and farrowing [40 g/fetus/day; based on McPherson et al. (2004)] from sow body weight at entering the farrowing stable. For each piglet, mother and foster sow were recorded, and for piglets that died during lactation date, body weight and cause of death (assessed by experienced staff of Pig Research Centre Raalte based on external appearance) were recorded. Date of first insemination, and whether a sow was pregnant or not after first insemination, was recorded for each sow. All data were recorded for both the litter preceding and following the dietary treatments.

Statistical analyses

Litter characteristics at birth, piglet mortality and weaning characteristics were analysed for sows with a weaning-to-insemination interval ≤ 7 days (127 of 137 sow cycles), that farrowed from first insemination cycle after weaning (117 of 127 sow cycles) and with > 4 piglets total born (115 of 117 sow cycles). Throughout the statistics and results sections, pre-weaning piglet mortality refers to mortality of live born piglets only.

Within the first 3 days after birth, 6% (118/1864) of the piglets (in 56% of the litters) were cross-fostered. Preliminary analyses showed that cross-fostered and non-cross-fostered piglets (which were still alive at day 4 after birth) had similar ($P > 0.05$) birth weights (absolute and relative to mean litter birth weight), age of death, body weight at death and mortality from days 4 - weaning. Furthermore, cross-fostered and non-cross-fostered litters had similar litter characteristics (number of piglets born, mean birth weight and uniformity parameters) based on both total born and live born piglets at birth. Finally, birth litters and foster litters had similar piglet mortality (days 0 - 3, days 4 - 7, days 8 - weaning and days 0 - weaning) and similar effects of treatment and parity on piglet mortality. Therefore, analyses regarding litter characteristics at birth and live born piglet mortality were based on birth litters. Analyses on weaning characteristics were based on foster litters.

Preliminary analyses showed that litter characteristics based on total born piglets and live born piglets were highly correlated ($r \geq 0.86$; $P < 0.0001$), and that relationships

between early live born piglet mortality and litter characteristics were comparable for litter characteristics based on total born piglets and live born piglets. Results are therefore presented for total born piglets only.

The continuous variables number of piglets (total born, live born, stillborn, weaned), mean birth weight of the litter and litter uniformity parameters (standard deviation (SD), coefficient of variation (CV), and percentage of piglets < 800 g) were analysed using the MIXED procedure of SAS 9.1 (SAS Inst. Inc, Cary, NC, USA). The binary variables (0/1) percentage of sows inseminated within 7 days after weaning, farrowing rate from first insemination after weaning, and percentage of litters with piglets < 800 g were analysed using the GLIMMIX procedure of SAS 9.1, using a binomial distribution and a logit link function. Piglet mortality (% mortality of live borns during days 0 - 3, days 4 - 7, days 8 - weaning and days 0 - weaning) was analysed at sow level using the GLIMMIX procedure of SAS 9.1, also using a binomial distribution and a logit link function; for each litter, the number of "trials" was defined as the total number of live born piglets per litter and the number of "events" was defined as the number of piglets that died per litter. For all these analyses (on sow level), model I was used: $Y_{ijkl} = \mu + T_i + P_j + B_k + T_i{*}P_j + e_{ijkl}$, where Y_{ijkl} = dependent variable, μ = overall mean, T_i = treatment (i = CON, WII, LAC + WII), P_j = parity after treatment (j = 2, 3 + 4, ≥ 5), B_k = batch (k = 1, 2, …, 14), $T_i{*}P_j$ = the interaction between treatment and parity, and e_{ijkl} = residual error. The factor sow was added as a random effect to model I (because 34 sows were used twice in the experiment). Preliminary analyses showed that sows of parity 5 - 7 and 8 - 12 did not differ in the number of total born (16.5 vs. 15.9 piglets) or stillborn piglets (1.5 vs. 2.2 piglets), mean birth weight (1.30 vs. 1.29 kg), birth weight CV (23.7 vs. 25.2%), percentage of piglets < 800 g (12.2 vs. 12.7%) and pre-weaning piglet mortality (days 0 - 3: 20.2 vs. 20.7%; days 0 - weaning: 34.0 vs. 28.2%). Therefore, for all analyses, parity was divided into three classes (2, 3 + 4, ≥ 5).

To analyse whether early mortality of light live born piglets was influenced by treatment or parity, piglet birth weight was divided into 2 classes (< 800 g and ≥ 800 g; and < 1 kg or ≥ 1 kg), and piglet mortality (from days 0 - 3) was analysed at piglet level using the GLIMMIX procedure of SAS 9.1, using a binomial distribution and a logit link function, and model II: $Y_{ijkl} = \mu + T_i + P_j + BW_k + T_i{*}BW_k + P_j{*}BW_k + e_{ijkl}$, where Y_{ijkl} = dependent variable, μ = overall mean, T_i = treatment (i = CON, WII, LAC + WII), P_j = parity after treatment (j = 2, 3 + 4, ≥ 5), BW_k = birth weight (k = < 800 g, ≥ 800 g; or k = < 1 kg, ≥ 1 kg), $T_i{*}BW_k$ = the interaction between treatment and birth weight, $P_j{*}BW_k$ = the interaction between

parity and birth weight, and e_{ijkl} = residual error. Both factors litter and sow were added as random effects to model II.

Piglet weaning weight and piglet growth during lactation were analysed on piglet level, using the MIXED procedure of SAS 9.1, and model III: $Y_{ijkl} = \mu + T_i + P_j + B_k + b_1 \text{nwean}_{ijkl} + T_i{*}P_j + e_{ijkl}$, where Y_{ijkl} = dependent variable, μ = overall mean, T_i = treatment (i = CON, WII, LAC + WII), P_j = parity after treatment (j = 2, 3 + 4, ≥ 5), B_k = batch (k = 1, 2, …, 14), b_1 = regression coefficient, nwean_{ijkl} = the number of weaned piglets in the litter (as a covariate), $T_i{*}P_j$ = the interaction between treatment and parity, and e_{ijkl} = residual error. Both factors litter and sow were added as random effects to model III. Additionally, piglet birth weight (as a covariate) and its interactions with treatment and parity were added to model III.

In all the models described above, the factors and interactions included in the model were tested for significance and stepwise omitted from the model if $P > 0.05$ (except for the factors treatment and parity). Bonferroni corrections were used for multiple comparisons.

Additionally, relevant covariates and their interactions with treatment and parity were added to model I (i) to check whether treatment and parity effects on litter characteristics at birth and live born piglet mortality were related to the number of total born piglets (therefore the MIXED procedure was used with the percentage of piglets that died per litter as the dependent variable; % mortality between days 4 - 7 and days 8 - weaning were transformed as ln(% + 1), because assumptions of normality were not fulfilled); (ii) to analyse relationships among litter characteristics; (iii) to analyse relationships between litter characteristics and early live born piglet mortality (therefore the MIXED procedure was used with the percentage of piglets that died within the first 3 days after birth per litter as the dependent variable); and (iv) to check whether relations between litter characteristics and live born piglet mortality from days 0 - 3 were related to the number of total born piglets. To test for possible interactions between the number of total born piglets, mean birth weight and birth weight uniformity regarding piglet mortality, number of total born piglets (< 17, 17 - 18 and ≥ 19 total born piglets; 35, 29, 36% of litters, respectively), mean birth weight (< 1.20 kg, 1.20 - 1.30 kg and ≥ 1.30 kg; 39, 26 and 35% of litters, respectively) and birth weight CV (< 21%, 21 - 27% and ≥ 27%; 33, 35 and 32% of litters, respectively) were divided into 3 classes and analysed as class variables.

Data are presented as means ± SE (overall means) or LSmeans ± SEM (effects of parity and treatment).

RESULTS

Lactation, weaning-to-insemination interval and farrowing rate

During the 41 ± 4 days of lactation preceding the dietary treatments, sows had an average estimated weight after farrowing of 225 ± 3 kg, an average weaning weight of 221 ± 3 kg and lost on average 4 ± 2 kg body weight (1.7 ± 0.7%).

In total, 92.7% (127/137) of the sows was inseminated within 7 days after weaning, with an average weaning-to-insemination interval of 4.2 ± 0.04 days. From these sows, 92.1% (117/127) farrowed from first insemination. The percentage of sows inseminated within 7 days and the percentage of those sows that farrowed from their first insemination were not affected by treatment (P = 0.21 and 0.54, respectively) or parity class (P = 0.70 and 0.60, respectively).

In Table 6.2 the litter characteristics at birth, live born piglet mortality during lactation and weaning characteristics for sows of different parity classes and treatments are presented.

Effect of parity

Litter characteristics at birth

Sows farrowed on average 17.4 ± 0.3 total born and 16.2 ± 0.3 live born piglets. The number of total and live born piglets was higher for parity 3 and 4 sows than for older sows, whereas parity 2 sows had an intermediate litter size (Table 6.2). The number of stillborn piglets increased with parity class (Table 6.2).

Mean piglet birth weights were on average 1.26 ± 0.02 kg for total born, 1.28 ± 0.02 kg for live born and 0.98 ± 0.03 kg for stillborn piglets. Mean piglet birth weights were not influenced by parity class (Table 6.2).

Litter uniformity of birth weights of total born piglets, i.e. SD, CV, and percentage of piglets < 800 g, were comparable for the parity classes (Table 6.2). However, after corrections for the number of total born piglets, birth weight CV (P = 0.05) and percentage of piglets < 800 g (P = 0.02) were higher for parity ≥ 5 sows (CV: 24.8%; % piglets < 800 g: 13.6%) than for parity 2 sows (CV: 21.3%; % piglets < 800 g: 6.2%), whereas parity 3 and 4 sows had an intermediate birth weight CV and percentage piglets < 800 g (CV: 23.2%; % piglets < 800g: 8.7%). The percentage of litters containing piglets < 800 g (61.9, 79.6 and 60.0 % of litters of parity 2, 3 + 4 and ≥ 5, respectively; P = 0.14) did not differ among parity classes.

Piglet mortality during lactation

Live born piglet mortality during lactation was 26.6 ± 1.5%; 65.5% of live born mortality occurred during the first 3 days after parturition, 13.2% during days 4 - 7 after parturition, and the remaining 21.3% after the first week of lactation, which is graphically shown in Figure 6.1a. Piglet mortality from days 0 - 3 and from days 4 - 7 increased with parity class (Table 6.2), which remained significant after correction for the number of total born piglets, but piglet mortality from days 8 - weaning did not differ among parity classes (Table 6.2).

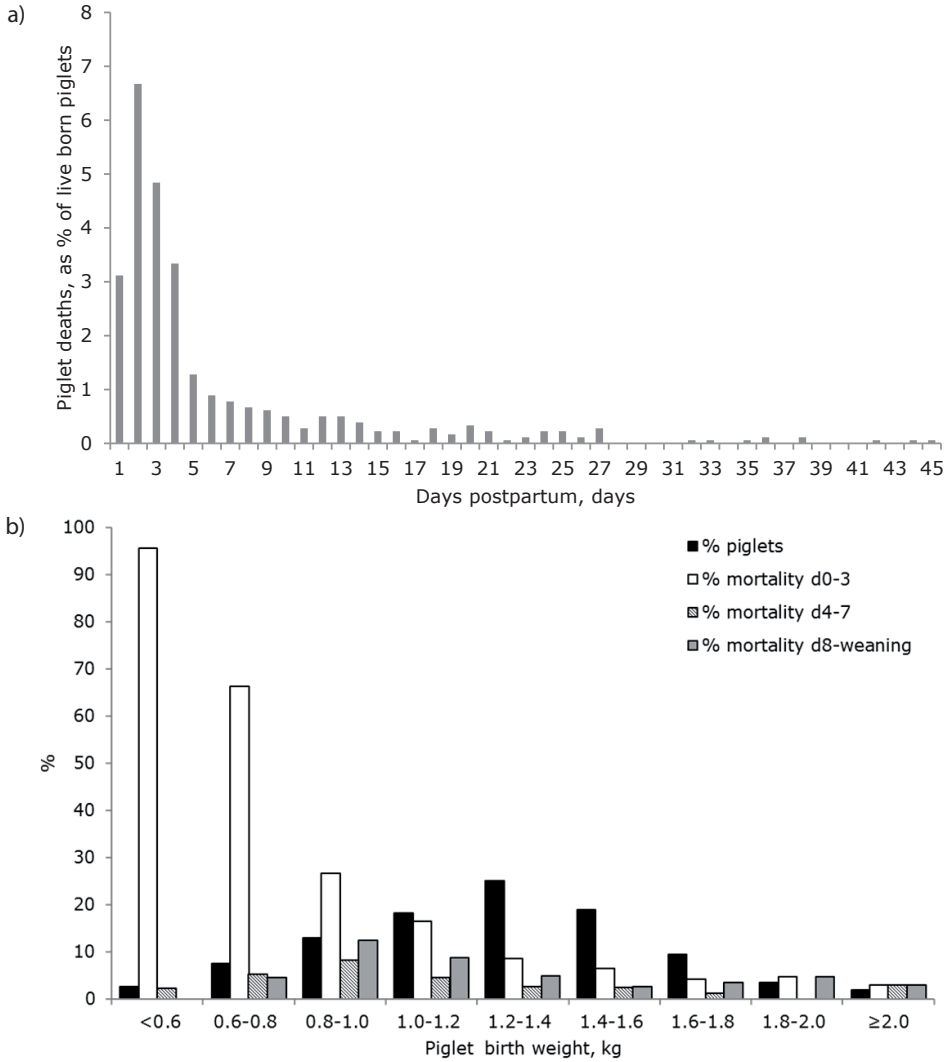

Figure 6.1 **a)** Distribution of live born piglet deaths during lactation; and **b)** Number of piglets and mortality of live born piglets during lactation (days 0 - 3, days 4 - 7 and days 8 - weaning) for different birth weight classes; in % of number of live born piglets (n = 1798 live born piglets in total).

Table 6.2 Litter characteristics at birth, live born piglet mortality during lactation and weaning characteristics for sows of different parity classes (2, 3 + 4, ≥ 5) and treatments (control sows, CON; sows fed extra sucrose and lactose during the weaning-to-insemination interval, WII; or sows fed extra sucrose and lactose during the last 2 weeks of preceding lactation and weaning-to-insemination interval; LAC + WII) (LSmeans ± SEM)

	Parity after treatment				Treatment				*P*-value[1]	
	2	3 + 4	≥ 5	SEM	CON	WII	LAC +WII	SEM	Parity	Treatment
Number of sow cycles[2]	21	44	50		34	42	39			
Parity after treatment	2.0	3.4	6.9		4.7	5.0	4.3			
Total born[3]										
Total born piglets, n	17.0[ab]	18.8[a]	16.3[b]	0.6	17.0	17.2	17.8	0.5	0.002	0.53
Mean birth weight, kg	1.27	1.23	1.29	0.04	1.28	1.29	1.23	0.03	0.30	0.48
SD birth weight, kg	0.27	0.29	0.30	0.01	0.29	0.28	0.28	0.01	0.14	0.58
CV birth weight, %	21.1	24.1	24.1	1.1	23.4	22.6	23.3	1.0	0.11[8]	0.79
Piglets < 800 g, %	5.7	10.4	12.3	1.9	8.4	7.9	12.2	1.9	0.09[8]	0.19
Litter weight, kg	21.3[ab]	22.7[a]	20.6[b]	0.6	21.3	21.5	21.6	0.6	0.02[9]	0.92
Live born										
Live born piglets, n	16.4[ab]	17.7[a]	14.7[b]	0.5	15.8	16.2	16.8	0.5	0.0002	0.34
Mean birth weight, kg	1.30	1.29	1.24	0.03	1.30	1.29	1.24	0.03	0.20	0.43
SD birth weight, kg	0.26	0.29	0.30	0.01	0.29	0.28	0.27	0.01	0.08	0.65
CV birth weight, %	20.3	23.8	23.3	1.1	22.6	22.1	22.6	1.0	0.07	0.92
Stillborn										
Stillborn piglets, n	0.4[a]	1.0[ab]	1.7[b]	0.3	1.1	1.0	1.0	0.3	0.01	0.98
Mean birth weight[4], kg	0.92	0.97	1.00	0.08	0.96	0.93	1.00	0.06	0.77	0.71

	Parity after treatment				Treatment				*P*-value[1]	
	2	3 + 4	≥ 5	SEM	CON	WII	LAC +WII	SEM	Parity	Treatment
Piglet mortality[5]										
Days 0 - 3, %	11.3[a]	17.6[ab]	20.6[b]	1.7	15.8	16.0	16.5	1.6	0.02[10]	0.96
Days 4 - 7, %	2.6[ab]	2.0[a]	5.5[b]	0.9	4.8	2.3	2.6	0.8	0.01[10]	0.05[11]
Days 8 - weaning, %	6.2	4.0	5.9	1.0	5.1[ab]	8.3[b]	3.4[a]	0.9	0.25	0.01[11]
Days 0 - weaning, %	20.9[a]	24.2[ab]	33.3[b]	2.6	27.2	27.2	23.1	2.5	0.01[10]	0.33
Weaning characteristics[6]										
Number of piglets weaned	12.5[a]	12.1[a]	10.1[b]	0.3	11.5	11.3	12.0	0.3	< 0.01	0.27
Piglet weaning weight[7], kg	11.9	11.7	12.0	0.2	11.9	11.8	11.9	0.2	0.64	0.82
Piglet growth during lactation[7], g/day	252	246	259	5	253	251	254	5	0.13	0.86

[1] Statistical significance; the treatment * parity interaction was never significant ($P > 0.05$). The effect of batch only stayed in the model when significant ($P \leq 0.05$).
[2] Sows with a weaning-to-insemination interval > 7 days (10 of 137 cycles), that did not farrow from first insemination cycle after weaning (10 of 127 cycles) and with < 4 total born piglets (2 of 117 cycles) were excluded.
[3] Excluding mummified piglets.
[4] Only for 60 sow cycles with stillborn piglets.
[5] Based on the number of live born piglets in the birth litter.
[6] Based on the number of weaned piglets in the foster litter.
[7] Corrected for the effect of the number of weaned piglets in the foster litter.
[8] The effect of parity became significant after addition of total born piglets as covariate to the model ($P = 0.05$ and 0.02 for CV and piglets < 800 g, respectively).
[9] The effect of parity became non-significant after addition of total born piglets as covariate to the model ($P = 0.54$).
[10] The effect of parity remained significant after addition of total born piglets as covariate to the model ($P = 0.01$, 0.01 and < 0.01 for days 0 - 3, days 4 - 7 and days 0 - weaning, respectively).
[11] The effect of treatment remained significant after addition of total born piglets as covariate to the model ($P = 0.01$ and 0.02 for days 4 - 7 and days 8 - weaning, respectively).
[ab] Within parity or treatment, values lacking a common superscript differ ($P \leq 0.05$).

From all live born piglet deaths, 40.2% was due to crushing, 18.5% due to low viability (weak piglets with a low birth weight), and remaining deaths (41.3%) were due to other (32.6%; e.g. leg problems, splay leg, insufficient milk intake or milk production by sow) or unknown reasons (8.7%).

Figure 6.1b shows the number and mortality of live born piglets for different birth weight classes. During the first 3 days after birth, mortality of light piglets at birth (< 800 g or < 1 kg) was significantly higher compared to heavier piglets at birth (LSmeans were 73.0 and 10.3% for piglets < 800 g and ≥ 800 g, respectively; $P < 0.001$; and 44.7 vs. 8.3% for piglets < 1 kg and ≥ 1 kg, respectively; $P < 0.001$), but mortality of light piglets was not influenced by parity class.

Weaning characteristics

Fifth or higher parity sows weaned less piglets than younger sows (Table 6.2). After correction for the number of weaned piglets, piglet weaning weight and piglet growth during lactation did not differ between parity classes. Both piglet weaning weight and piglet growth during lactation were strongly related to piglet birth weight (regression coefficients were 5.05 kg/kg for weaning weight, $P < 0.01$; and 97.7 (g/day)/kg for piglet growth, $P < 0.01$); these relationships were independent of parity class.

Effect of pre-mating insulin-stimulating sow diets

Litter characteristics at birth

Litter size (total, live and stillborn), mean piglet birth weight and birth weight uniformity were not affected by the treatments (Table 6.2), nor was the percentage of litters containing piglets < 800 g (61.8, 66.7 and 74.4% of CON, WII and LAC + WII litters, respectively; $P = 0.56$).

Piglet mortality during lactation

Piglet mortality from days 0 - 3 and days 0 - weaning was not affected by the treatments (Table 6.2), nor was the mortality of light piglets (< 800 g or < 1 kg) during the first 3 days after birth. However, piglet mortality from days 4 - 7 tended to be higher in CON litters than in WII litters (Table 6.2), whereas piglet mortality from days 8 - weaning was higher in WII than in LAC + WII litters (Table 6.2).

Weaning characteristics

The number of piglets weaned, piglet weaning weight and piglet growth during lactation were not influenced by the treatments (Table 6.2).

Birth litter characteristics and early piglet mortality

Table 6.3 shows the relationships amongst litter characteristics and between litter characteristics and live born piglet mortality during the first 3 days after birth. Litter characteristics at birth and live born piglet mortality during the first 3 days after birth were affected by parity class, but treatment effects were never significant. Therefore, all relationships presented were corrected for parity class effects, but not for treatment effects.

Relationships amongst litter characteristics

Several relationships existed among the different litter characteristics (Table 6.3). For example, for each additional piglet (total born) in a litter, mean birth weight decreased with 40 g, CV of birth weight increased with 0.76%, and the percentage of piglets < 800 g increased with 1.48% (Table 6.3). Furthermore, for every 100 g decrease in mean piglet birth weight in a litter, CV of birth weights increased with 1.58%, and percentage of piglets < 800 g increased with 3.8% (Table 6.3). Figure 6.2a and 6.2b illustrate the relationships between the number of total born piglets and mean birth weight and birth weight CV, respectively, for different parity classes.

Table 6.3 Regression coefficients (βs) amongst litter characteristics and between litter characteristics of total born piglets and live born piglet mortality during the first 3 days after birth[1]

Litter characteristic (total born)[2]	Litter characteristic (total born)[2]				Piglet mortality during the first 3 days after birth[3], %
	Total born, n	Mean birth weight, kg	SD of birth weight, kg	CV of birth weight, %	
Total born, n	-	-	-	-	1.47***
Mean birth weight, kg	- 0.040***	-	-	-	- 30.99***[4]
SD of birth weight, kg	0.002	0.0001	-	-	37.49
CV of birth weight, %	0.76***	- 15.82***	73.24***	-	1.08***[4]
Piglets < 800 g, %	1.48***	- 38.01***	39.65*	1.31***	0.58**[4]

[1] Relationships were corrected for the effect of parity class (2, 3 + 4, ≥ 5). The interaction between parity class and litter characteristic was never significant ($P > 0.05$).
[2] Based on total born piglets in birth litter.
[3] In % based on number of live born piglets in birth litter.
[4] Relationship remained significant after correction for the number of total born piglets.
* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

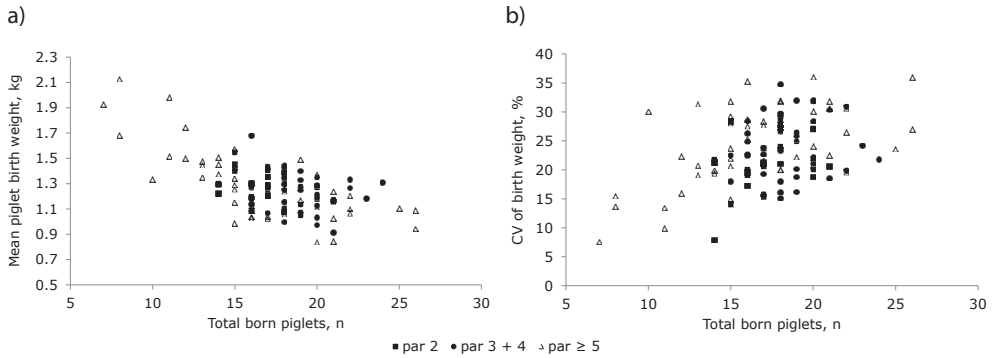

Figure 6.2 Relationship between the number of total born piglets and **a)** Mean piglet birth weight and **b)** CV of birth weights (based on birth litter), for different parity classes.

To find out whether the CV of birth weight was directly related to the number of total born piglets, or indirectly via effects on mean birth weight, analyses were done with either number of total born piglets or mean birth weight as a covariate, or with both covariates together. The model including both total born piglets and mean birth weight showed the best model fit (*P*-values were 0.26 and < 0.01 for number of total born and mean birth weight, respectively).

Relationships between litter characteristics and early piglet mortality

Live born piglet mortality during the first 3 days after birth was strongly related to the number of total born piglets; i.e. for each additional total born piglet, piglet mortality increased with 1.47% (Table 6.3). Piglet mortality from days 0 - 3 was also strongly related to mean piglet birth weight (+ 3% mortality for every 100 g decrease in mean birth weight), the CV of birth weights (+ 1.08% mortality for every % increase in CV) and the percentage of piglets < 800 g (+ 0.58% mortality for every % increase in percentage piglets < 800g; Table 6.3); these relationships (regression coefficients and *P*-values) did not change after correction for the number of total born piglets (the corresponding *P*-values for total number of piglets born were 0.26, 0.02 and 0.06 in the models for mean birth weight, CV and percentage of piglets < 800 g, respectively). In Figure 6.3a and 6.3b relationships between piglet mortality and mean birth weight and birth weight CV, respectively, are illustrated for different litter sizes.

The interactions among the number of total born piglets, mean birth weight and birth weight CV regarding piglet mortality were never significant. Comparing different models

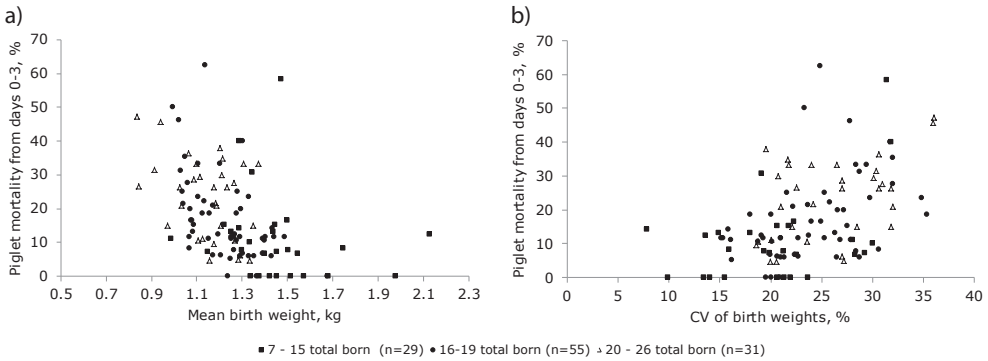

Figure 6.3 Relationship between **a)** Mean birth weight and **b)** CV of birth weights of birth litter with live born piglet mortality during the first 3 days after birth (based on birth litter), for different number of total born piglets classes.

for piglet mortality, using the number of total born piglets, mean birth weight and birth weight CV as class variables, the model including all variables together showed the best model fit (with *P*-values of 0.21, 0.04 and 0.01 for number of total born piglets, mean birth weight and birth weight CV, respectively). Figure 6.4 shows the effect of mean birth weight and birth weight CV on piglet mortality.

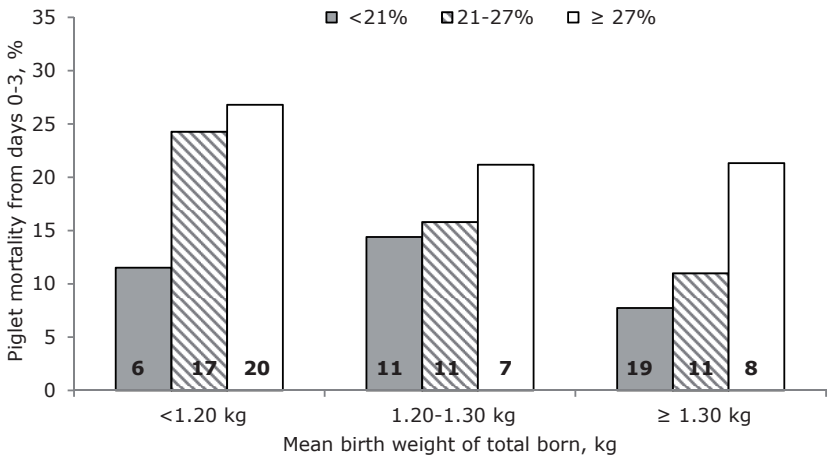

Figure 6.4 Effect of both mean piglet birth weight (< 1.20, 1.20 - 1.30 and ≥ 1.30 kg) and birth weight CV (< 21, 21 - 27, and ≥ 27%) of total born piglets on live born piglet mortality during the first 3 days after birth (based on birth litter); the number of observations representing each bar is given within the bars (LSmeans from the model including mean birth weight ($P = 0.05$), birth weight CV ($P = 0.003$) and their interaction ($P = 0.50$); when the interaction was excluded from the model, *P*-values were < 0.01 for both mean birth weight and birth weight CV).

DISCUSSION

We studied relationships between piglet birth weight, piglet uniformity and pre-weaning piglet mortality, and effects of pre-mating insulin-stimulating sow diets, in organic sows with large litters. In these large organic litters, birth weight, both individual and mean birth weight, and birth weight uniformity appeared strongly related to piglet mortality during lactation. Piglet uniformity and live born piglet mortality were both affected by parity, but not by pre-mating insulin-stimulating diets.

Causes of death were recorded by experienced staff of Pig Research Centre Raalte, based on external appearance. Because the distinction between stillborn piglets and piglets dying soon after birth without autopsy can be difficult (Vaillancourt et al. 1990), live born piglet mortality might be underestimated in this study (because piglets that died soon after birth may have been misclassified as stillborn piglets). Furthermore, Vaillancourt et al. (1990) reported that deaths due to crushing/trauma can be accurately classified by external appearance, but recordings of other causes of deaths (especially 'low viability') should be interpreted with caution.

With an average litter size of 17.4 total born piglets, litter sizes in these organic sows were high. Leenhouwers et al. (2011) reported larger litter sizes in organic farms compared to conventional farms in The Netherlands, which is most likely related to the longer lactations [on average 41.9 and 25.5 days in organic and conventional farms, respectively; Leenhouwers et al. (2011)]. Longer lactation lengths, i.e. an increase from 25 to 40 days of lactation, is associated with higher subsequent litter sizes (Xue et al. 1993; Dewey et al. 1994; Tummaruk et al. 2000b). The biological mechanisms through which longer lactations lead to higher litter sizes are not fully elucidated yet, but may involve the gradual restoration from a negative energy balance towards a more positive energy balance with prolonged lactation [related to a gradual reduction in suckling frequency over time coinciding with an increase in piglet creep feed intake; e.g. Puppe and Tuchscherer (2000)]. Conventional sows are in a negative energy balance during their 3 - 4 week lactation, which suppresses follicle development and – as a consequence – suppresses subsequent fertility [for review see e.g. Quesnel (2009)]. In the current study, sows lost on average only 4 ± 2 kg body weight (1.7 ± 0.7%) during their 6 week lactation, which suggests that they turn into a positive energy balance during the last weeks of lactation, and therefore probably have a better follicle development at weaning compared to conventionally weaned sows. An improved follicle development at weaning may lead to higher ovulation rates and/or

reduced embryonic mortality [for review see e.g. Quesnel (2009)], which will be reflected in larger litter sizes at birth.

Despite the larger litters born, organic sows wean on average fewer piglets than conventional sows [10.3 vs. 11.2 piglets; Leenhouwers et al. (2011)], due to a higher pre-weaning piglet mortality [25.5 vs. 12.4% in organic and conventional sows, respectively; Leenhouwers et al. (2011)]. Pre-weaning piglet mortality (excluding stillborns) was on average 26.6% in this study. Major causes of death were crushing (40.2%) and low viability (18.5%). Although loose housing of sows is often associated with more deaths due to more crushing compared to sows confined in farrowing crates (Blackshaw et al. 1994; Mortensen et al. 1994; Marchant et al. 2000), the percentage of deaths due to crushing in this study (40.2%) is comparable to percentages reported for sows kept in farrowing crates: 39% (Mortensen et al. 1994) and 48% (Marchant et al. 2000). More recent studies showed that loose housing does not necessarily lead to a higher pre-weaning mortality and more crushing than farrowing crates (Cronin et al. 2000; Weber et al. 2007, 2009; Pedersen et al. 2011), and that causes of mortality are comparable for loose housed sows and sows in farrowing crates (Pedersen et al. 2011). Therefore, the high pre-weaning piglet mortality in the current organic litters is probably mainly related to the large litter sizes. Larger litter sizes at birth are associated with lower and less uniform birth weights (Quesnel et al. 2008; this study). In the currently studied litters, mean piglet birth weights were only 1.26 kg and average birth weight CV was 23.5%, which is relatively low and high, respectively, compared to previous studies in smaller litters (Milligan et al. 2002; Damgaard et al. 2003; Van den Brand et al. 2006, 2009; Quesnel et al. 2008). Both birth weight and birth weight uniformity are important factors for survival of piglets (Milligan et al. 2002; Quiniou et al. 2002; this study). Additionally, larger litters generally lead to longer farrowing durations and more variable birth intervals, resulting in more asphyxia during farrowing and subsequently more stillbirths and more weak piglets at birth which are at greater risk of dying (Marchant et al. 2000; Pedersen et al. 2006; Weber et al. 2009). Other factors that may have contributed to the high pre-weaning mortality in these organic litters may be a lower milk output due to organic feeds [with lower quality protein compared to conventional sow feeds; Sundrum (2001)] and/or a lower nursing frequency due to larger litters (Rydhmer et al. 2005). O'Reilly et al. (2006) reported that longer lactations (≥ 27 days vs. 21 - 26 days) are associated with a higher pre-weaning piglet mortality. However, in the current study, only minor piglet losses occurred after 27 days of lactation (piglet mortality until day 27 of lactation was on average 26.1% vs. 26.6% until weaning). Therefore, we suggest that the high pre-weaning

piglet mortality in organic sows is mainly due to the large litter sizes at birth, resulting in lower and less uniform birth weights and weaker piglets, probably resulting in more losses due to different types of reasons.

In these large organic litters, strong relationships were found amongst litter characteristics (total number born, mean birth weight, CV of birth weight and percentage of piglets < 800 g), and between these litter characteristics and early live born piglet mortality, which is in accordance with previous results in conventional – smaller – litters (Milligan et al. 2002; Quiniou et al. 2002; Quesnel et al. 2008). When mean birth weight or birth weight uniformity parameters were included simultaneously in the model with total number born, *P*-values for the effect of total number born increased or even became non-significant. This suggests that the unfavourable effect of litter size on birth weight uniformity and on piglet mortality, is mainly an indirect effect through effects of litter size on mean birth weight and birth weight uniformity. In contrast to findings of Milligan et al. (2002), mean birth weight and birth weight uniformity did not interact regarding (early) piglet mortality in this study. The lack of a significant interaction may be related to a lower number of observations in the current study and the strong negative relation between mean birth weight and birth weight CV, which resulted in only few observations with either low mean birth weight and low birth CV or high mean birth weight and high birth weight CV. We conclude that both piglet birth weight and birth weight uniformity affect pre-weaning piglet mortality in (organic) sows with large litters, as they do in conventional smaller litters.

Besides the unfavourable effects of large litter sizes at birth on pre-weaning piglet mortality, larger litters probably also have lower and less uniform weaning weights [e.g. Milligan et al. (2002)], and reduced post-weaning performance of pigs [e.g. Quiniou et al. (2002); Berard et al. (2008); Beaulieu et al. (2010)], related to the lower piglet birth weight and uniformity. Strong effects of piglet birth weight on weaning weight and growth during lactation were confirmed in the current study. It seems plausible, therefore, that ongoing selection for litter size will result in lighter and less uniform piglets at birth, with presumably unfavourable consequences for piglet performance during and after lactation. However, despite the strong relations found between litter size and mean birth weight and birth weight uniformity, and between these litter characteristics and early piglet mortality, still a large variation exists in piglet birth weight, piglet uniformity and piglet mortality among sows with equal litter sizes (see Figure 6.2 and 6.3). This suggests that there may be opportunities to improve birth weight, uniformity and survival of piglets, with a simultaneous increase in litter size.

Higher parity sows (≥ 5) farrowed fewer piglets (total and live born), had more stillborn piglets, less uniform piglets and a higher percentage of light piglets (< 800 g) compared to younger sows. Furthermore, pre-weaning piglet mortality was highest in sows of higher parity (≥ 5).

Generally, litter size is reported to increase with parity, reaching the highest level at parities 3 - 5 and either reaching a plateau (Dewey et al. 1995; Hughes, 1998; Quesnel et al. 2008) or slowly declining (Koketsu et al. 1999; Tummaruk et al. 2000a; Kongsted and Hermansen, 2009; Hoving et al. 2011) thereafter. Older parity sows often have less uniform litters and a higher proportion of light piglets (Milligan et al. 2002; Damgaard et al. 2003; Quesnel et al. 2008), but whether this is a parity effect as such or whether this is related to the parity effect on litter size is not clear. In the present study, litter size of older parity sows (≥ 5) was on average 2.5 piglets less than that of parity 3 and 4 sows, but birth weight CV and proportion of light piglets were comparable. However, after correction for the number of total born piglets, older parity sows (≥ 5) had a higher birth weight CV and a higher proportion of light piglets than younger sows. This suggests that litter uniformity is also partly affected by parity as such, independent of litter size effects. The question arises whether the observed decrease in piglet uniformity in older sows could be related to changes in follicle development and follicle quality with ageing, as reported for example in human [e.g. Broekmans et al. (2009)].

The observed increase in piglet mortality with an increased parity, both in number of stillbirths [also seen by Leenhouwers et al. (1999), Lucia et al. (2002), Milligan et al. (2002), Canario et al. (2006), and Quesnel et al. (2008)] and live born deaths [also seen by Marchant et al. (2000), Milligan et al. (2002), Weber et al. (2007, 2009)] can be related to (i) the generally less uniform litters with more light piglets in older sows; (ii) a hampered and prolonged birth process in older sows, due to e.g. excessive fatness and/or reduced uterine muscle tone (Randall, 1972; Zaleski and Hacker, 1993); and (iii) a reduced and more variable functionality and accessibility of teats in older sows (Fraser and Thompson, 1986; Dyck et al. 1987).

To conclude, the current results indicate that both piglet uniformity and pre-weaning piglet mortality (stillborn and live born mortality) in organic sows with large litters are affected by parity as such, in addition to parity effects on litter size.

In contrast to findings in conventional sows (Van den Brand et al. 2006, 2009), the insulin-stimulating diets during different stages of the pre-mating period did not improve piglet birth weight or piglet uniformity, nor reduced piglet mortality during lactation in these

organic sows. The insulin-stimulating diet in the current experiment consisted of sucrose and lactose, which has comparable insulin-stimulating effects as the dextrose and lactose diet (Wientjes et al. 2012a), as used by Van den Brand et al. (2009). In conventional sows, a reduction in insulin levels induced by a strong catabolic state during lactation is shown to suppress follicle development at weaning and subsequent fertility [as reviewed by e.g. Quesnel (2009)]. This could explain the findings that in conventional sows, which are in a catabolic state until weaning, insulin-stimulating diets during lactation and/or the weaning-to-estrus interval can improve follicle development and subsequent piglet uniformity (Van den Brand et al. 2006, 2009). The currently studied organic sows most likely switched to an anabolic state during the last weeks of lactation, related to the longer lactations (as discussed before). In an anabolic state insulin levels are not suppressed anymore, thus the longer lactations probably resulted in a better follicle development at weaning compared to conventional sows, as reflected by the larger litter sizes. Therefore, insulin-stimulating diets during the pre-mating period may be beneficial for follicle development and subsequent piglet uniformity, but only so in sows with a compromised follicle development at weaning, due to e.g. a severe catabolic state until weaning.

CONCLUSION

Organic litters have low mean birth weights and a high birth weight CV, associated with the large number of piglets born, and a high pre-weaning piglet mortality. In these large organic litters, birth weight, both individual and mean birth weight, and birth weight uniformity are strongly related to live born piglet mortality during lactation. Piglet uniformity decreases and piglet mortality increases with parity as such, in addition to litter size effects. Insulin-stimulating diets before mating do not improve piglet uniformity and mortality in organic sows with large litters, which suggests that pre-mating insulin-stimulating diets may only be beneficial for follicle development and subsequent piglet uniformity in sows with a compromised follicle development.

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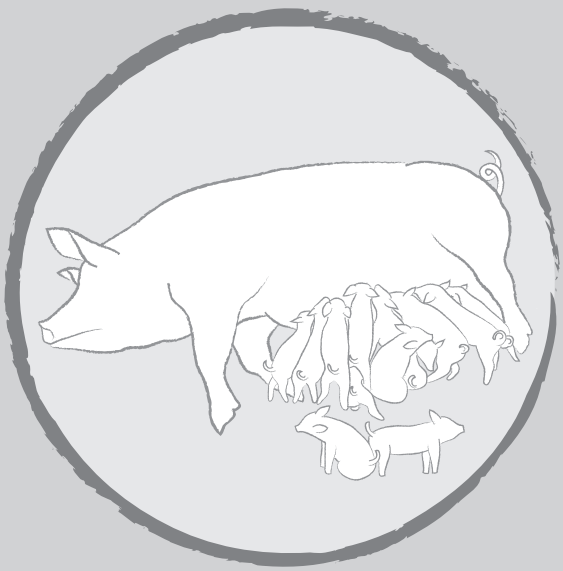
Chapter 7

Piglet birth weight and litter uniformity: Effects of weaning-to-pregnancy interval and body condition changes in sows of different parities and crossbred lines

JGM Wientjes[1]
NM Soede[1]
EF Knol[2]
H van den Brand[1]
B Kemp[1]

[1] Adaptation Physiology Group, Department of Animal Sciences, Wageningen University, Wageningen, The Netherlands

[2] IPG, Institute for Pig Genetics BV, Beuningen, The Netherlands



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ABSTRACT

Piglet birth weight and litter uniformity were studied in sows of different parities and crossbred lines in relation to (i) the weaning-to-pregnancy interval (WPI); and (ii) sow body condition changes (in BW and backfat thickness) during lactation and pregnancy in sows with a short WPI (≤ 7 days). At the IPG research farm, individual piglet birth weights and sow body condition (BW and backfat thickness at farrowing and weaning) were measured of 949 TOPIGS20 and 889 TOPIGS40 sows with > 4 total born piglets, inseminated between 2003 and 2011. In all analyses, mean piglet birth weight and birth weight SD and CV were corrected for total number born. Total number born was higher in sows with a WPI of 8 to 21 days (+ 1.2 piglets; n = 72) and > 21 days (+ 0.7 piglets; n = 182) compared to sows with a WPI ≤ 7 days ($P < 0.01$; n = 1584). Mean piglet birth weight was not affected by WPI. Birth weight SD (- 23 g) and CV (- 1.7%) were lower in sows with a WPI > 21 days compared to sows with a WPI ≤ 7 days ($P < 0.01$). Effects of WPI were independent of sow parity. Effects of body condition changes in sows with a WPI ≤ 7 days were studied separately in TOPIGS20 sows inseminated between 2006 and 2011 (n = 808) and in TOPIGS40 sows inseminated between 2003 and 2008 (n = 747). Sow body condition loss during lactation was not related to subsequent total number born or mean piglet birth weight. Only in TOPIGS20 sows, more BW loss during lactation was related to higher subsequent birth weight SD (β = 0.83 g/kg, $P < 0.01$; β = 1.62 g/%, $P < 0.01$), and more backfat loss during lactation was related to higher subsequent birth weight SD (β = 5.11 g/mm, $P < 0.01$) and CV (β = 0.36 %/mm, $P < 0.01$), independent of sow parity. Sow BW increase during pregnancy was negatively related to total number born (TOPIGS20: β = - 0.06 and - 0.05 piglet/kg BW increase for parity 2 ($P < 0.01$) and 3 and 4 ($P < 0.01$), respectively; TOPIGS40: β = - 0.04 piglet/kg BW increase ($P < 0.01$) independent of sow parity). Sow BW increase during pregnancy was positively related to birth weight SD (TOPIGS20: β = 0.63 g/kg BW increase ($P = 0.01$) independent of sow parity). Sow body condition increase during pregnancy was not related to mean piglet birth weight. To conclude, this study shows that litter uniformity is compromised by severe sow body condition loss during lactation and improved in sows with a prolonged WPI. These effects are likely related to (insufficient) restoration of follicle development.

INTRODUCTION

Important factors affecting pre-weaning piglet survival are piglet birth weight and litter uniformity (Milligan et al. 2002; Quiniou et al. 2002). Both piglet birth weight and litter uniformity are associated with litter size and parity (Milligan et al. 2002; Quesnel et al. 2008; Wientjes et al. 2012c), but there may be other factors involved.

Body condition loss during lactation can delay estrus, through inhibition of GnRH secretion, or decrease subsequent pregnancy rates and litter sizes, through insufficient restoration of follicle development, affecting ovulation rate and embryo quality (Quesnel 2009). Insufficient restoration of follicle development may also increase developmental variation within the pre-ovulatory follicle pool, which may affect embryo survival and development (Pope et al. 1990; Xie et al. 1990a, b; Zak et al. 1997b) and luteal development (Wientjes et al. 2012a, b), and is expected to ultimately affect piglet birth weight and litter uniformity (Wientjes et al. 2012a, b). A prolonged interval from weaning to successful pregnancy (through delaying estrus or pregnancy failure), on the other hand, may allow follicle restoration after weaning and thereby affect subsequent litter characteristics.

Besides body condition changes during lactation, body condition changes during pregnancy may also influence piglet birth weight and litter uniformity. Campos et al. (2012), for example, reviewed that sow feed intake during pregnancy can affect piglet birth weight and may affect litter uniformity.

We thus hypothesize that sow body condition change may be another factor influencing piglet birth weight and litter uniformity. We studied litter characteristics at birth, with a focus on piglet birth weight and litter uniformity, in sows of different parities and crossbred lines in relation to (i) the weaning-to-pregnancy interval (WPI); and (ii) sow body condition changes (in BW and backfat thickness) during lactation and pregnancy in sows with a short WPI (≤ 7 days).

MATERIALS AND METHODS

General

From the experimental farm of the Institute for Pig Genetics (IPG) in Beilen (The Netherlands), data on reproductive performance and sow body condition were available for two commercial crossbred sow lines: TOPIGS20 (Large White 1 x Landrace cross) and

TOPIGS40 (Synthetic x Large White 2; the Synthetic is a Piëtrain derived dam line, selected on maternal traits for over 30 years), inseminated between January 2003 and February 2011. Care and treatment of animals was according to Dutch animal welfare legislation.

Housing and feeding

Sows were kept individually in farrowing crates from 9.6 ± 3.1 days before farrowing until weaning. Before farrowing, sows were gradually adapted to a standard (commercially available) lactation diet (ca. 9.6 MJ NE/kg; 140 g/kg CP; 6.7 g/kg ileal digestible lysine) fed twice daily at 0700h and 1500h. During lactation, the feeding level gradually increased to a maximum at day 14 of lactation of 7 kg/day (primiparous sows) or 8 kg/day (multiparous sows), independent of number of nursed piglets [see Bergsma and Hermesch (2012)]. Piglets were cross fostered to equalize litter sizes.

After weaning, sows were housed in individual crates and fed to satiety twice daily at 0730h and 1530h with a (commercially available) flush diet (ca. 9.2 MJ NE/kg; 135 g/kg CP; 6.1 g/kg ileal digestible lysine). From one day after weaning sows were checked for estrus twice daily (0800h and 1500h) using a mature boar. All sows were inseminated each day of estrus with a dose of semen (2 x 10^9 sperm cells) of one of six boar lines (boar lines were alternately used, using two boar lines per farrowing batch).

From approximately two days after last insemination, sows were housed in groups of approximately 20 sows and fed a standard (commercially available) pregnancy diet (ca. 9.0 MJ NE/kg; 125 to 130 g/kg CP; 4.3 to 4.7 g/kg ileal digestible lysine) supplied in feeding stations, according to the following scheme for gilts, parity 2 and older sows, respectively: wk 0 to 4: 2.52, 2.66 and 2.80 kg/day; wk 5 to 11: 2.25, 2.38 and 2.50 kg/day; wk 12 to 15: 2.88, 3.04 and 3.20 kg/day; and wk 16: 2.52, 2.66 and 2.80 kg/day. At approximately 24 days after insemination, sows were checked for pregnancy using transcutaneous ultrasonography.

Measurements and calculations

Sow BW and ultrasound backfat thickness were measured at entering the farrowing crate and at weaning. For each sow, farrowing date, weaning date, date of first insemination (of each insemination cycle), whether the sow was pregnant or not after an insemination cycle, number of piglets born alive, number of piglets born dead, and number of piglets weaned were recorded per parity. Piglets (alive and stillborn) were weighed individually at birth.

The number of total born piglets was calculated from number of piglets born alive and born dead (excl. mummified piglets). Weaning-to-pregnancy interval (WPI) was defined as the interval from weaning to insemination for the insemination cycle that resulted in a successful pregnancy. Sow BW at farrowing was calculated as BW at entering the farrowing crate, adjusted for weight of the fetuses, placentas and intra-uterine fluid according to Bergsma et al. (2009). Sow BW at weaning was adjusted for water content of mammary glands, as described by Bergsma et al. (2009). Sow BW loss during lactation was derived as corrected BW at farrowing minus corrected BW at weaning. Sow BW increase during pregnancy was derived as corrected BW at farrowing minus corrected BW at previous weaning.

Data selection

Only litters from sows with > 4 total born piglets were included in the analyses. Sows that nursed a second litter as a foster sow within the same lactation were also excluded. First parity litters were excluded from analyses regarding the effects of WPI and body condition changes (because in first parity sows BW and backfat thickness was only measured at farrowing). Four data subsets were used for the analyses: *TOTAL≤7d*, *WPI*, *T20/06-11* and *T40/03-08*.

TOTAL≤7d

Dataset including 2128 litters of sows inseminated between 2003 and 2011, with a WPI ≤ 7 days, and that farrowed from the first insemination cycle after weaning.

WPI

To study the effect of a prolonged WPI on litter characteristics at birth, the litters of sows with a WPI > 7 days (n = 136) and repeat breeders (n = 118) were added to TOTAL≤7d.

T20/06-11 and *T40/03-08*

Because the two different sow lines were not evenly distributed over the years (number of sows inseminated in 2003 – 2011 were 29, 0, 0, 52, 149, 274, 351, 306 and 56 for TOPIGS20, respectively, and 101, 172, 273, 204, 107, 54, 0, 0 and 0 for TOPIGS40, respectively), two sub-datasets were made from *TOTAL≤7d*: ***T20/06-11:*** this dataset only included the litters of TOPIGS20 sows that were inseminated between 2006 and 2011 (n = 808, excl. first parity litters); and ***T40/03-08:*** this dataset only included the litters of TOPIGS40 sows that were inseminated between 2003 and 2008 (n = 747, excl. first parity litters).

Statistical analyses

Response variables were litter characteristics at birth, based on total born piglets: total number of piglets born, litter weight at birth, mean birth weight, within-litter SD of birth weight, within-litter CV of birth weight, the percentage of piglets < 1000 g and > 1800 g. Normality was checked by examining skewness and kurtosis of variables and model residuals, with a skewness and kurtosis between - 2 and 2 considered normal. For the analysis of percentage of piglets < 1000 g and > 1800 g the square root arcsine transformation for proportions was used to meet these assumptions of normality.

Data were analyzed using the MIXED procedure of SAS 9.2 (SAS Inst. Inc., Cary, NC, USA). Results are presented as LSM ± SEM, unless otherwise stated. For all MIXED analyses, first interaction terms were tested and removed from the model when not significant ($P > 0.05$), except for the interaction between parity and sow line.

Effects of parity and sow line on litter characteristics at birth were analyzed using dataset *TOTAL≤7d* with a mixed model, including parity (1, 2, 3 and 4, ≥ 5), sow line (TOPIGS20, TOPIGS40) and its interaction as fixed factors, and the factors sow (using standard variance components covariance structure, type = VC) and farrowing batch [using a first-order autoregressive covariance structure, type = AR(1)] as random effects (MODEL 1). For mean birth weight, SD and CV of birth weight and percentage of piglets < 1000 g and > 1800 g, the number of total born piglets and its interactions with parity class and sow line were always included as additional covariates. Preliminary analyses showed that boar line, analyzed as an additional random effect, did not significantly affect subsequent litter characteristics ($P > 0.10$), and that the addition of boar line to the models did not improve model fit. Therefore, we did not include the specific effect of boar line in our statistical analyses.

Effects of a prolonged WPI on litter characteristics at birth were analyzed in dataset *WPI* using MODEL 1, except that WPI class (≤ 7 days, 8 to 21 days, > 21 days + repeat breeders) and its interactions with parity and sow line were added to the model. Preliminary analyses showed that litter characteristics at birth did not differ between repeat breeders (n = 118) and sows with a WPI > 21 days (and not recorded as repeat breeder; n = 64) and therefore both groups were combined.

An overview of lactation and pregnancy characteristics for *T20/06-11* and *T40/03-08* is given in Table 7.1. Sow body condition change variables (BW and backfat loss during lactation; BW and backfat increase during pregnancy) were arbitrarily divided into three classes as follows: 20% lowest observations, 60% average observations and 20% highest observations.

Table 7.1 Overview of previous lactation and pregnancy characteristics (only for sows with a weaning-to-pregnancy interval ≤ 7 days, that farrowed from first insemination cycle after weaning, and with > 4 total born piglets)

	T20/06-11[1]			T40/03-08[2]		
Item	n	Avg ± SD	Range	n	Avg ± SD	Range
Parity	808[3]	3.2 ± 1.2	2 to 7	747[4]	4.5 ± 2.0	2 to 9
Previous lactation						
Lactation length, days	808	26.3 ± 3.1	17 to 39	747	26.4 ± 3.8	17 to 45
BW at weaning, kg	752	186 ± 31	121 to 278	691	209 ± 36	116 to 305
BW loss during lactation, kg	747	21.5 ± 12.0	- 26 to 61	687	13.7 ± 12.4	- 33 to 55
BW loss during lactation, %	747	10.4 ± 5.9	- 14 to 27	687	6.2 ± 5.8	- 17 to 29
Backfat thickness at weaning, mm	773	14.6 ± 3.0	7.0 to 25.0	705	14.6 ± 3.8	6.5 to 29.0
Backfat loss during lactation, mm	772	4.4 ± 2.0	- 1.5 to 11.5	704	3.3 ± 2.1	- 7.5 to 12.5
Number of piglets weaned, n	808	11.6 ± 1.2	5 to 14	747	10.3 ± 1.4	5 to 14
Piglet weaning weight, kg	799	7.6 ± 1.3	3.7 to 14.7	747	8.0 ± 1.5	3.5 to 15.5
Litter weight at weaning, kg	799	88.4 ± 15.1	29 to 143	747	81.6 ± 17.1	31 to 155
Pregnancy						
BW increase during pregnancy, kg	643	40.3 ± 14.0	- 20 to 79	645	30.5 ± 14.4	- 31 to 79
BW at farrowing, kg	698	228 ± 28	161 to 309	700	238 ± 31	160 to 344
Backfat thickness increase during pregnancy, mm	665	4.3 ± 2.5	- 6.0 to 14.0	659	3.6 ± 2.7	- 8.5 to 14.5
Backfat thickness at farrowing, mm	698	19.0 ± 3.7	7.5 to 31.5	700	18.1 ± 4.5	9.0 to 38.0

[1] TOPIGS20 sows that were inseminated between 2006 and 2011 (first parity sows were excluded).
[2] TOPIGS40 sows that were inseminated between 2003 and 2008 (first parity sows were excluded).
[3] 281 sows of parity 2, 410 sows of parity 3 and 4, 117 sows of parity ≥ 5.
[4] 147 sows of parity 2, 250 sows of parity 3 and 4, 350 sows of parity ≥ 5.

Effects of body condition change variables on litter characteristics at birth were first analyzed in the datasets *T20/06-11* and *T40/03-08*, using a mixed model, including parity (2, 3 and 4, ≥ 5), a sow body condition change variable (using similar classes in both datasets) and its interaction as fixed factors, and the factors sow (using standard variance components covariance structure, type = VC) and farrowing batch [using a first-order autoregressive covariance structure, type = AR(1)] as random effects (MODEL 2). For mean birth weight, SD and CV of birth weight, and percentage of piglets < 1000 g and > 1800 g, the number of total born piglets was always included as additional covariate. When effects of sow body condition change variables on litter characteristics at birth had a *P*-value ≤ 0.15, and litter characteristics were either gradually increasing or decreasing from the 20% lowest observations class to the 20% highest observations class, additional analyses were done with the sow body condition change variables analyzed as covariates instead of class variables, using MODEL 2. Sow BW increase during pregnancy was strongly confounded with parity class (resulting in parity class x sow BW increase during pregnancy class combinations with very few observations), and was therefore only analyzed as a covariate (taking into account the interaction between parity class and sow BW increase). Preliminary analyses showed that lactation length, divided into: ≤ 24 days (20% lowest observations), 25 to 27 days (60% average observations) and ≥ 28 days (20% highest observations), did not affect subsequent litter characteristics ($P > 0.10$). Additionally, preliminary analyses excluding sows with lactation lengths of < 23 days (n = 57 (7%) in T20/06-11 and n = 82 (11%) in T40/03-08) and > 29 days (n = 79 (10%) in T20/06-11 and n = 96 (13%) in T40/03-08) gave similar results regarding effects of body condition changes on subsequent litter characteristics. Therefore, lactation length was not further considered in the analyses.

For the datasets *T20/06-11* and *T40/03-08*, Pearson correlations between sow body condition change variables during previous lactation and pregnancy were calculated within each parity class.

RESULTS

Effects of parity and sow line

The total number of piglets born and litter weight at birth increased with parity and were higher in TOPIGS20 sows than in TOPIGS40 sows (Table 7.2). Mean piglet birth weight was lower in first parity sows than in older sows, and higher in TOPIGS20 sows than in

Table 7.2 Effects of parity and sow line on litter characteristics of total born piglets at birth (only for sows with a weaning-to-pregnancy interval ≤ 7 days, that farrowed from first insemination cycle after weaning, and > 4 total born piglets, n = 2128) (LSM ± SEM)

Factor	Class	n	Total number of piglets born	Litter weight at birth, kg	Mean birth weight[1], g	SD of birth weight[1], g	CV of birth weight[1], %	Piglets < 1000 g[1,2], %	Piglets > 1800 g[1,2], %
Parity	1	544	12.5[a]	16.3[a]	1275[a]	246	19.4	14.3	3.3
	2	428	12.7[a]	18.3[b]	1429[b]	294	21.0	10.8	10.8
	3 and 4	669	14.2[b]	20.0[c]	1460[c]	309	21.6	10.8	14.0
	≥ 5	487	14.3[b]	19.6[c]	1442[bc]	322	23.1	12.4	14.8
	SEM		0.2	0.2	25	4	0.3	1.6	2.2
	P-value		< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Sow line	TOPIGS20	1217	14.0[b]	19.0[b]	1430[b]	283	20.7	11.5	10.9
	TOPIGS40	911	12.9[a]	18.1[a]	1373[a]	303	21.9	12.6	9.4
	SEM		0.2	0.2	26	4	0.3	1.6	2.3
	P-value		< 0.01	< 0.01	0.01	< 0.01	< 0.01	0.26	0.31
Parity x sow line interaction	*P*-value		0.58	0.18	0.43	< 0.01	0.02	0.046	0.02

[abc] Within parity or sow line, means lacking a common superscript differ ($P \leq 0.05$).

[1] Corrected for the effect of total number born.

[2] For the analyses, the square root arcsine transformation for proportions was used. Results shown are transformed back into percentages.

TOPIGS40 sows (Table 7.2; corrected for total number born). For SD and CV of birth weight and the percentage small and large piglets, an interaction between parity class and sow line existed (Table 7.2; corrected for total number born). In general, results in first parity sows were similar for both sow lines. Results from first parity sows deviated from higher parity sows, and in these higher parity sows results differed between sow lines (Figure 7.1).

Mean birth weight and litter uniformity parameters were strongly affected by total number born. For each additional piglet, mean birth weight decreased by 41 g ($P < 0.01$), SD and CV of birth weight increased by 4 g ($P < 0.01$) and 0.8% ($P < 0.01$), respectively, and the percentage of small (< 1000 g) piglets increased by 1.9% ($P < 0.01$); these relationships were similar among parities and sow lines (i.e. interactions with parity class or sow line were not significant). The relationship between total number born and the percentage of large (> 1800 g) piglets differed among parities (parity class x total number born interaction $P <$

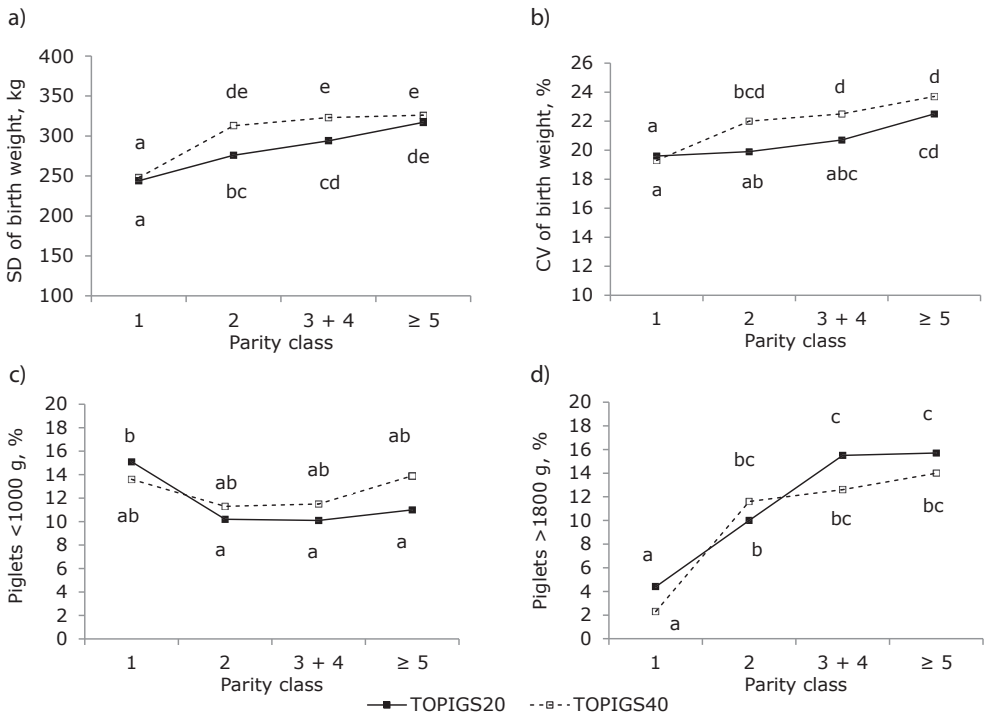

Figure 7.1 Interaction (LSM) between parity class and sow line (corrected for total number born) for **a)** SD of birth weight of total born (interaction $P < 0.01$; pooled SEM = 6); **b)** CV of birth weight of total born (interaction $P = 0.02$; pooled SEM = 0.5); **c)** % piglets < 1000 g of total born (interaction $P = 0.046$; pooled SEM = 1.8); and **d)** % piglets > 1800 g of total born (interaction $P = 0.02$; pooled SEM = 2.5); [abcde] Within each graph, LSM without a common superscript differ ($P \leq 0.05$).

0.01; βs were - 1.7 ($P < 0.01$), - 3.2 ($P < 0.01$), - 3.2 ($P < 0.01$) and - 3.3 %/piglet ($P < 0.01$) for parity 1, 2, 3 and 4 and ≥ 5, respectively) and sow lines (sow line x total number born interaction $P < 0.01$; βs were - 2.6 ($P < 0.01$) and - 3.6 %/piglet ($P < 0.01$) for TOPIGS20 and TOPIGS40, respectively).

Effects of a prolonged WPI

Total number of piglets born was higher for sows with a prolonged WPI (+ 1.2 piglets and + 0.7 piglets for sows with a WPI of 8 to 21 days and > 21 days, incl. repeat breeders, respectively) than for sows with a WPI ≤ 7 days (Table 7.3). Litter weight at birth was higher for sows with a 8 to 21 days WPI (+ 0.9 kg) than for sows with a WPI ≤ 7 days (Table 7.3). Mean birth weight and the percentage of small and large piglets were not affected by WPI (Table 7.3; corrected for total number born). The SD and CV of birth weight were lower for sows with a WPI > 21 days, including repeat breeders (SD - 23 g; CV - 1.7%) than for sows with a WPI ≤ 7 days (Table 7.3; corrected for total number born).

Table 7.3 Effect of weaning-to-pregnancy interval (WPI) on litter characteristics of total born piglets at birth (LSM ± SEM)

Item	WPI ≤ 7 days	WPI 8 to 21 days	WPI > 21 days + repeat breeders	SEM	*P*-value[1]
Number of litters, n	1584	72	182		
TOPIGS20	837	22	90		
TOPIGS40	747	50	92		
Parity	3.9[b]	4.2[b]	3.5[a]	0.2	< 0.01
Total number born, n	13.7[a]	14.9[b]	14.4[b]	0.3	< 0.01
Litter weight at birth, kg	19.3[a]	20.2[b]	19.9[ab]	0.3	< 0.01
Mean birth weight[2], g	1428	1438	1431	17	0.83
SD of birth weight[2], g	310[b]	291[ab]	287[a]	7	< 0.01
CV of birth weight[2], %	22.2[b]	20.8[ab]	20.5[a]	0.5	< 0.01
Piglets < 1000 g[2,3], %	11.7	10.7	10.4	0.9	0.15
Piglets > 1800 g[2,3], %	11.9	12.6	11.5	1.7	0.83

[ab] Within a row, means without a common superscript differ ($P \leq 0.05$).

[1] The interactions between parity class and WPI, and between sow line and WPI were never significant ($P > 0.05$) and therefore removed from the model.

[2] Corrected for the effect of total number born. When not corrected for total number born, LSM for WPI ≤ 7 days, WPI 8 to 21 days and WPI > 21 days + repeat breeders were 1438, 1398 and 1416 g, respectively, for mean birth weight ($P = 0.12$), 308[b], 295[ab], and 289[a] g, respectively, for SD of birth weight ($P < 0.01$), 22.0, 21.7 and 21.0%, respectively, for CV of birth weight ($P = 0.14$), 11.3, 12.0 and 11.0%, respectively, for % piglets < 1000 g ($P = 0.77$), and 12.5, 9.7 and 10.4%, respectively, for % piglets > 1800 g ($P = 0.10$).

[3] For the analyses, the square root arcsine transformation for proportions was used. Results shown are transformed back into percentages.

Effects of body condition changes in sows with a WPI ≤ 7 days

Sow body condition change variables (BW and backfat loss during previous lactation; BW and backfat increase during pregnancy) that were significantly ($P \leq 0.05$) related to litter characteristics at birth (as a class variable, as a covariate or both) are shown in Table 7.4 for the dataset *T20/06-11*, and mentioned below for the dataset *T40/03-08*.

Sow body condition loss during previous lactation

T20/06-11

Sow BW loss during lactation was positively related to subsequent litter weight at birth ($P = 0.05$ as class variable and $P = 0.03$ as covariate for body weight loss in kg; Table 7.4), SD of birth weight ($P = 0.04$ as class variable and $P < 0.01$ as covariate for body weight loss in kg; $P = 0.02$ as class variable and $P < 0.01$ as covariate for body weight loss in %; Table 7.4), and percentage of piglets > 1800 g ($P < 0.01$ as covariate for body weight loss either in kg or in %; Table 7.4). Backfat loss during lactation was positively related to subsequent SD of birth weight ($P = 0.04$ as class variable and $P < 0.01$ as covariate; Table 7.4) and CV of birth weight ($P = 0.05$ as class variable and $P < 0.01$ as covariate; Table 7.4). These effects were independent of sow parity.

T40/03-08

Sow BW loss during lactation (either in kg or in %) and backfat loss during lactation did not affect subsequent litter characteristics at birth ($P > 0.05$).

Sow body condition increase during pregnancy

T20/06-11

Sow BW increase during pregnancy was negatively related to total number of piglets born in parity 2 to 4 sows (β = - 0.06 piglet/kg, $P < 0.01$ for parity 2; β = - 0.05 piglet/kg, $P < 0.01$ for parity 3 and 4). Sow BW increase during pregnancy was negatively related to litter weight (β = - 0.029 kg/kg, $P < 0.01$), and positively related to SD of birth weight ($\beta = 0.63$ g/kg, $P = 0.01$) and percentage of piglets > 1800 g ($\beta = 0.17$ %/kg, $P < 0.01$), independent of sow parity. Sow backfat thickness increase during pregnancy was negatively related to total number of piglets born ($P < 0.01$ as covariate; Table 7.4) and litter weight ($P = 0.04$ as class variable and $P < 0.01$ as covariate; Table 7.4), independent of sow parity.

Table 7.4 Effects of sow body condition change variables[1] on litter characteristics of total born piglets at birth in TOPIGS20 sows that were inseminated in 2006 to 2011 (*T20/06-11*; n = 808)

Body condition change variable	Litter characteristic	20% lowest	60% average	20% highest	SEM	*P*-value	Regression coefficient	*P*-value
BW loss during lactation, kg		≤ 8	8 to 28	> 28				
	Litter weight, kg	19.3	19.6	20.2	0.3	0.05	0.022	0.03
	SD of birth weight[2], g	281[a]	299[ab]	307[b]	7	0.04	0.83	< 0.01
	Piglets > 1800 g[2], %	7.0	9.0	10.4	1.6	0.14	0.15	< 0.01
BW loss during lactation, %		≤ 3.5	3.5 to 13	> 13				
	SD of birth weight[2], g	279[a]	299[ab]	307[b]	8	0.02	1.62	< 0.01
	Piglets > 1800 g[2], %	7.6	8.9	10.4	1.6	0.15	0.30	< 0.01
Backfat loss during lactation, mm		≤ 2	2 to 5	> 5				
	SD of birth weight[2], g	285[a]	297[ab]	310[b]	7	0.04	5.11	< 0.01
	CV of birth weight[2], %	20.6[a]	21.6[ab]	22.4[b]	0.6	0.05	0.36	< 0.01
Backfat increase during pregnancy, mm		≤ 1.5	1.5 to 5.5	> 5.5				
	Total number born, n	14.9	14.2	14.0	0.3	0.09	- 0.13	< 0.01
	Litter weight, kg	20.5[b]	19.7[ab]	19.3[a]	0.4	0.04	- 0.17	< 0.01

Only for sows with a weaning-to-pregnancy interval ≤ 7 days, that farrowed from first insemination cycle after weaning, and > 4 total born piglets.

[ab] Within a row, means without a common superscript differ ($P \leq 0.05$).

[1] First analyzed as a class variable (divided into 3 classes, presented as LSM ± SEM). When effects had a *P*-value ≤ 0.15 and effects seemed linear, additional analyses were done with sow body condition change variables as covariates (presented as regression coefficients).

[2] Corrected for the effect of total number born.

T40/03-08

Sow BW increase during pregnancy was negatively related to total number of piglets born (β = - 0.04 piglet/kg, $P < 0.01$) and litter weight (β = - 0.03 kg/kg, $P < 0.01$), independent of sow parity. Sow backfat thickness increase during pregnancy did not affect litter characteristics at birth.

Correlations between sow body condition changes during lactation and pregnancy

Within each parity class, sow BW increase during pregnancy was positively correlated to BW loss during previous lactation (both in kg and in %; *T20/06-11*: $r \geq 0.37$, $P < 0.01$; *T40/03-08*: $r \geq 0.30$, $P < 0.05$) and backfat loss during previous lactation (*T20/06-11*: $r \geq 0.19$, $P < 0.01$; *T40/03-08*: $r \leq 0.11$, $P > 0.05$). Within each parity class, sow backfat thickness increase during pregnancy was positively correlated to backfat loss during previous lactation (*T20/06-11*: $r \geq 0.23$, $P < 0.01$; *T40/03-08*: $r \geq 0.24$, $P < 0.01$).

DISCUSSION

Sow body condition loss during lactation is reported to prolong the weaning-to-estrus interval, or decrease subsequent pregnancy rates and litter sizes (Zak et al. 1997a; Van den Brand et al. 2000; Prunier et al. 2003; Thaker and Bilkei, 2005). This is the first study showing that also litter uniformity can be compromised by sow body condition loss during the previous lactation.

In sows with a regular WPI (≤ 7 days), sow body condition loss during lactation was negatively related to subsequent litter uniformity. For example, an increase in backfat loss during lactation from ≤ 2 mm to > 5 mm resulted in + 25 g in birth weight SD and + 1.8% in birth weight CV. Insufficient restoration of follicle development, as a consequence of severe body condition loss during lactation, may increase developmental variation within the pre-ovulatory follicle pool (Zak et al. 1997b). Xie et al. (1990b) demonstrated that the less developed embryos within a litter at day 12 of pregnancy represent the later maturing oocytes (from later-ovulating/smaller follicles), indicating that developmental variation in follicle and oocyte development may be reflected in developmental variation in zygote and early embryo development (Pope et al. 1990; Xie et al. 1990a, b). Van der Lende et al. (1990) made plausible that within-litter variation in embryonic development at day 35 of pregnancy

is representative for the within-litter variation in piglet birth weight. Additionally, there is increasing evidence that embryo survival and development and progesterone secretion (luteal function) during early pregnancy are influenced by pre-mating conditions (such as feeding level or metabolic profiles during lactation and weaning-to-estrus interval), rather than post-mating conditions [such as feeding level or metabolic profiles during early pregnancy; e.g. Zak et al. (1997a); Ashworth et al. (1999a, b); Hoving (2012); Wientjes et al. (2012a, b)]. It thus seems likely that effects of body condition loss during lactation on subsequent piglet uniformity are related to insufficient restoration of follicle development and increased developmental variation within the pre-ovulatory follicle pool, which may be reflected in compromised development and uniformity of embryos and placentas, and compromised luteal development, and thereby ultimately affecting litter uniformity.

A prolonged WPI (> 7 days) improved subsequent litter sizes (+ 0.7 to 1.2 piglets) and litter uniformity at birth (- 19 to 23 g in birth weight SD; - 1.4 to 1.7% in birth weight CV), possibly related to a longer recovery after weaning, and thereby restoration of follicle development (and uniformity). Previous studies reported that litter size was reduced in sows with a WPI of 7 to 12 days (exact range varies among studies and parities) compared to sows that had a shorter (≤ 6 days) or longer (> 12 days) WPI (Dewey et al. 1994; Vesseur et al. 1994; Le Cozler et al. 1997; Poleze et al. 2006). This dip in litter size was attributed to carry over effects of compromised follicle and oocyte development during lactation or suboptimal timing of insemination. The increase in litter size with an increase in WPI beyond 12 days was suggested to be related to recovery from body condition loss during lactation (Vesseur 1997; Poleze et al. 2006), and may thus be related to follicle restoration or the occurrence of post-weaning follicle turnover (Van Leeuwen 2011). In our study, due to the limited number of sows with a WPI > 7 days, sows with a WPI ranging between 8 and 21 days were analyzed as one group, and found to farrow 1.2 more piglets in their subsequent litter than sows with a WPI ≤ 7 days. The majority of these sows (74%) had a WPI of 12 to 21 days. Sows with a WPI > 21 days included repeat breeders and sows with an extended weaning-to-estrus interval (including sows in which first estrus was not detected). The total group of sows with a WPI > 21 days farrowed more piglets and more uniform litters than sows with a short WPI (≤ 7 days). Skipping the first estrus after weaning and inseminating at the second estrus in primiparous sows is known to result in up to 2.5 more piglets born in the subsequent litter (Clowes et al. 1994; Vesseur 1997; Werlang et al. 2011), and in large-scale multi-farm analyses repeat breeders are reported to farrow 0.4 to 0.6 piglets more compared to sows that farrowed from first insemination

after weaning (Tummaruk et al. 2001; Hoving, 2012). Although previous studies showed that the increase in litter size after skipping the first estrus or in repeat breeders is most evident in young sows [parity 2 and 3; Tummaruk et al. (2001); Hoving (2012)], in our study the beneficial effects of a prolonged WPI on litter size seemed independent of sow parity; although there were limited numbers of sows with a prolonged WPI. Because sows with a WPI > 7 days in our study had improved litter sizes and improved litter uniformity compared to sows with a short WPI (≤ 7 days), we suggest that most of these sows either had a lactational estrus or experienced post-weaning follicle turnover.

Although effects of pre-mating conditions, such as body condition loss during lactation and length of WPI, on litter uniformity may seem marginal, Wientjes et al. (2012c) reported that for every percent increase in birth weight CV, early piglet mortality increases by 1.08%. Thus the 1.4 to 1.8% increase in birth weight CV may correspond with an estimated 1.5 to 1.9% increase in piglet mortality. Besides an increase in piglet mortality, reduced birth weight uniformity is also associated with more variable weaning weights, resulting in increased labor and complicated slaughter management (Milligan et al. 2002).

Besides effects of pre-mating conditions, we also found relationships between body condition increase during pregnancy and litter characteristics at birth in sows with a short WPI. Sow BW increase and backfat increase during pregnancy were negatively related to litter size, litter weight, and litter uniformity. Quesnel et al. (2008) also reported a negative relationship between backfat thickness gain during pregnancy and litter uniformity. It should be noted that sow body development and litter development are competing nutritional demands during pregnancy; sows gestating larger litters, therefore, were likely unable to invest as much energy into their own body development compared to sows gestating smaller litters, given the higher priority of litter demands and a fixed feed intake (Lewis and Bunter 2011). Sow body condition increase during pregnancy was positively correlated to body condition loss during previous lactation, and litter uniformity was compromised by severe body condition loss during previous lactation. Thus, effects of body condition increase during pregnancy on subsequent litter characteristics might be carry-over effects of sow body condition loss during previous lactation.

Mean piglet birth weights were not affected by the WPI, nor by sow body condition changes during lactation or pregnancy. Litter birth weight (h^2 = 0.13 - 0.16) and mean piglet birth weight (h^2 = 0.30 - 0.50) have a higher heritability than litter uniformity [SD and CV h^2 < 0.10; Högberg and Rydhmer (2000); Hermesch et al. (2001); Damgaard et al. (2003); Wolf

et al. (2008); Kapell et al. (2011)], indicating a strong maternal genetic effect on litter and piglet birth weights.

Litter characteristics at birth were affected by litter size and sow parity. Litter size effects on mean piglet birth weight and litter uniformity are comparable with previous studies (Quiniou et al. 2002; Quesnel et al. 2008; Wientjes et al. 2012c), and independent of sow parity and sow line. Parity effects on litter characteristics at birth are also in accordance with previous studies (Damgaard et al. 2003; Quesnel et al. 2008; Wientjes et al. 2012c) and indicate that, in addition to the parity effect on litter size, mean piglet birth weight is highest in parity 3 and 4 sows, and litter uniformity decreases with parity, as a result of an increase in both the percentage of small piglets and the percentage of large piglets in older sows.

TOPIGS20 sows farrowed more piglets, that were heavier and more uniform compared to TOPIGS40 sows, and effects of sow body condition changes on litter characteristics at birth were substantially more pronounced in the TOPIGS20 sows inseminated between 2006 and 2011 than in the TOPIGS40 sows inseminated between 2003 and 2008. This may indicate that (negative) consequences of severe body condition loss during lactation for subsequent fertility may differ between sow lines, or have changed over the years, or both.

To conclude, this is the first study showing that litter uniformity can be compromised by severe sow body condition loss during lactation, and that litter uniformity is improved in sows with a prolonged WPI. These effects are likely related to (insufficient) restoration of follicle development. These results indicate that severe body condition loss of sows during lactation should be prevented, or otherwise the WPI could be prolonged (allowing follicle restoration and turnover), in order to assure good litter uniformity, and thereby piglet survival, in the subsequent litter.

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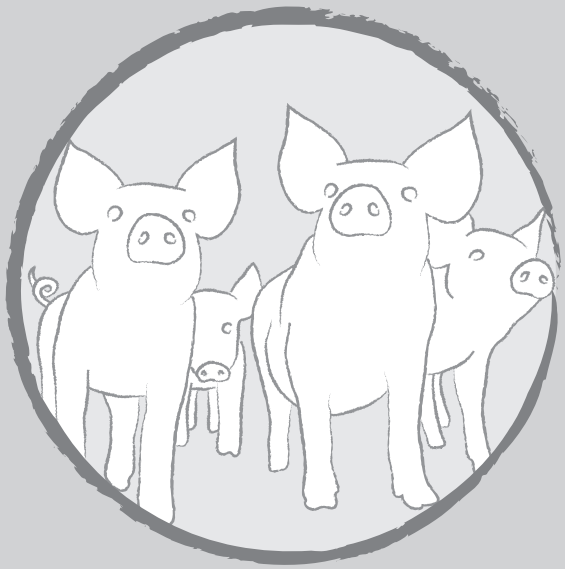
Chapter 8

General discussion



The main hypothesis of this thesis was that piglet birth weight and litter uniformity are already (partly) determined in the pre-mating period (as thoroughly described in Chapter 1). More specifically, it was hypothesized that pre-mating plasma insulin and IGF-1 levels affect follicle and oocyte development and uniformity, and thereby subsequent embryo development and uniformity and luteal development, subsequent placental and fetal development and uniformity and finally piglet birth weight and litter uniformity. Therefore, aims of this thesis were (i) to study effects of insulin-stimulating diets during the weaning-to-estrus interval on plasma insulin and IGF-1 levels, follicle development and uniformity, and consequences for embryo, fetal and placental development and uniformity and luteal development at different stages of pregnancy in sows; and (ii) to study effects of pre-mating conditions related to sow metabolic state during lactation and after weaning on subsequent piglet birth weight and litter uniformity.

In the following paragraphs the results obtained in this thesis will be discussed. In paragraph 8.1, first the importance of piglet birth weight and litter uniformity for pre-weaning piglet survival and the origin of within-litter birth weight variation, including effects of litter size and parity are discussed. In paragraph 8.2, effects of pre-mating conditions related to sow metabolic state during lactation and after weaning on subsequent piglet birth weight and litter uniformity are discussed. In paragraph 8.3, results of the physiological studies with insulin-stimulating diets during the weaning-to-estrus interval are discussed. General conclusions and recommendations are given in paragraph 8.4.

8.1 PIGLET BIRTH WEIGHT AND LITTER UNIFORMITY

8.1.1 Importance for pre-weaning piglet survival

Previous studies showed that in sow populations with average litter sizes of 11.2 total born piglets (Damgaard et al. 2003), 12.5 total born piglets (Quiniou et al. 2002) or 9.5 - 11.8 total born piglets (Milligan et al. 2002b), piglet birth weight and litter uniformity are important factors for pre-weaning piglet survival. Results of this thesis confirm that also in (organic) sows farrowing larger litters of 17.4 ± 0.3 total born piglets, piglet birth weight and litter uniformity are strongly related to piglet survival during lactation (Chapter 6). Regression coefficients indicate that for every 100 g increase in mean piglet birth weight, live-born piglet survival during the first 3 days after birth increases with 3.1%, whereas for every 1% reduction in within-litter birth weight CV, early piglet survival increases with 1.1%;

regression coefficients for live-born piglet survival during the whole (6-week) lactation period were 3.3% and 1.1%, respectively. This indicates that even marginal improvements in piglet birth weight or litter uniformity can have substantial effects on piglet survival.

It remains debatable whether beneficial effects of litter uniformity on piglet survival are mainly due to a lower number of low birth weight piglets (with a low survival chance) in more uniform litters, or are also due to better survival chances of low birth weight piglets in more uniform litters (due to a lower competitive disadvantage). Results of this thesis confirm that low litter uniformity at birth, i.e. a high birth weight CV, is strongly associated with lower mean birth weights and more low birth weight piglets (Table 8.1).

In observational studies, as in Chapter 6 as well as studies of Milligan et al. (2002a, b), the relation between litter uniformity and piglet survival is strongly confounded by the fact that litters with a low uniformity are often larger litters (with more competitors; Table 8.1) with more low birth weight piglets (with a low survival chance; Table 8.1), and from older parity sows [with more variable access to functional teats; Vasdal and Andersen (2012)]. Based on these observational studies, the conclusion that low litter uniformity in itself results in low survival can not be drawn. Experimental studies on the effect of litter uniformity on piglet survival, using mixing of litters to create uniform and variable litters with standardized litter sizes and numbers of low birth weight piglets within a litter, gave inconsistent results (Milligan et al. 2001a, b; English and Bilkei 2004). In small litters (8 - 9 piglets), consisting of 4 - 5 low birth weight piglets (~ 1 kg) and an equal number of slightly heavier (~ 1.2 - 1.6 kg) or much heavier (> 1.6 kg) littermates, survival of low birth

Table 8.1 Pearson correlations amongst CV of birth weight (as a measure for litter uniformity) and other litter characteristics (based on total born)

	CV of birth weight, %	
Litter characteristic	Chapter 6[1]	Chapter 7[2]
Total born, n	0.41***	0.38***
Mean birth weight, g	- 0.56***	- 0.41***
Piglets < 800 g, %	0.69***	n.a.
Piglets < 1000 g, %	0.64***	0.60***

[1] Organic Topigs20 sows with 41 ± 4 day lactations and 17.4 ± 3.3 total born piglets (n = 115); adapted from Chapter 6.
[2] Conventional Topigs20 and Topigs40 sows with 26 ± 4 day lactations and 13.5 ± 3.0 total born piglets (n = 2128); adapted from Chapter 7.
*** $P < 0.001$; n.a., not available.

weight piglets, as well as their ability to suckle successfully (as indicated by the number of successful or missed nursings and the frequency and duration of teat disputes), was not affected by the weight of their littermates (Milligan et al. 2001a, b; English and Bilkei 2004). In larger litters of 11 - 12 piglets, English and Bilkei (2004), but not Milligan et al. (2001a, b), found a higher mortality and lower ability to suckle successfully of low birth weight piglets when raised with much heavier littermates (19% mortality) compared to slightly heavier (12% mortality) or low birth weight littermates (9% mortality). All three studies found overall a higher frequency of missed nursings and teat disputes in large litters compared to small litters, which indicates more competition in large litters, likely due to a relative decrease in available number of functional teats (Milligan et al. 2001a, b; English and Bilkei 2004). Together, these studies seem to indicate that littermate weight, and thus litter uniformity, has more impact on survival of low birth weight piglets in larger litters when resources are more limited. In the – much larger – litters of 16.2 ± 0.3 live born piglets from Chapter 6, pre-weaning mortality of live born piglets with a low birth weight (< 1.0 kg) indeed tended to be lower in more uniform litters (54% in 56 litters with a birth weight CV < 22%) than in less uniform litters (65% in 59 litters with a high birth weight CV ≥ 22%; $P = 0.09$, after corrections for the random effect of litter; based on birth litters; Figure 8.1). It can be concluded that the beneficial effect of a high litter uniformity on piglet survival is primarily due to a lower number of low birth weight piglets in more uniform litters, and may also be due to higher survival chances of low birth weight piglets in more uniform litters, especially in large litters when the number of piglets exceeds the number of functional teats.

8.1.2 The origin of within-litter birth weight variation

The possible origin of within-litter birth weight variation is thoroughly described in Chapter 1, and will only be summarized here. Placental size is an important determinant of fetal growth and largely fixed at day 35 of pregnancy. Uniformity in placental size among littermates at day 35 of pregnancy, therefore, will be reflected in subsequent fetal growth and litter uniformity [based on Van der Lende et al. (1990)]. Uniformity in placental size is directly related to uniformity in embryo development at elongation and onset of implantation at ~ day 12 of pregnancy, because the more developed embryos start to elongate earlier and occupy a more than equal share of the uterine space, ultimately resulting in a larger placenta (Geisert and Schmitt 2002; Vallet et al. 2009). Indications exist that early embryo uniformity reflects follicle and oocyte uniformity (Pope 1988; Pope et

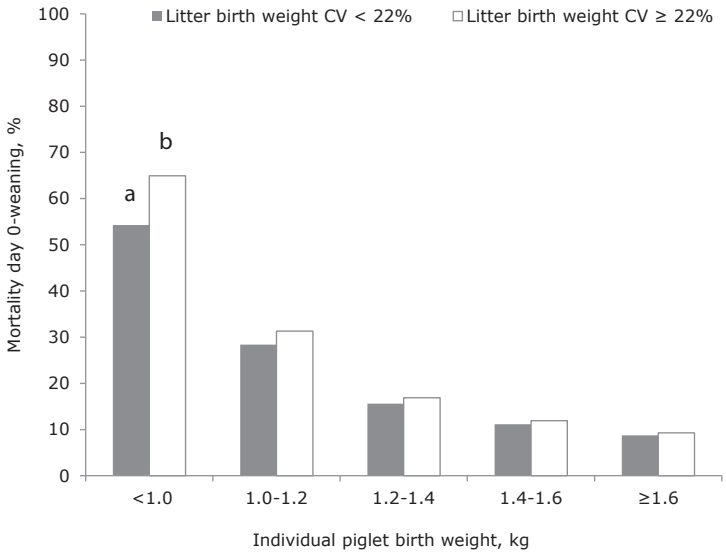


Figure 8.1 Mortality of live born piglets during lactation for different birth weight classes (in % of number of live born piglets) for litters with low (< 22%) or high (≥ 22%) birth weight CV (based on live born piglets in birth litter); ab bars with different superscript differ $P < 0.10$ (corrected for the random effect of birth litter); adapted from Chapter 6.

al. 1990; Xie et al. 1990a, b). Within-litter piglet weight variation at birth, therefore, is the consequence of very early existence of within-litter variation in early embryo development, which likely reflects variation in follicle and oocyte development.

Within-litter ranking of size of embryos or weight of fetuses, thus, remains more or less the same throughout pregnancy, and a high within-litter variation in early embryo development will result in a high within-litter variation at term. However, several mechanisms may modulate within-litter variation during pregnancy.

Mechanisms that may add within-litter variation during pregnancy

Embryos enter the uterus within ~ 2 - 3 days after fertilization when they are at the ~ 4-cell stage of development (Dziuk 1985). Between day 7 and 12 of pregnancy, the embryos migrate from the oviductal end of the uterine horn and redistribute themselves over the full length of both uterine horns by uterine motility (Pope et al. 1982; Dziuk 1985), although slightly more embryos will stay in the horn of origin than migrating to the other horn (Dziuk 1985). An uneven or disproportional spacing and distribution of embryos through the length of the uterine horns may interfere with the (genetic) growth potential of the

embryos, and thereby add additional within-litter variation in development, because a portion of the uterine horn with a disproportional high embryo density may not provide sufficient uterine space to support maximum growth of these embryos at a later stage of pregnancy. Dziuk (1985, 1992), for example, stated that embryo density is highest in the central portion of the uterus (near the cervix) and decreases towards the tips of the uterine horns (Dziuk 1992), and reported that the available uterine space for each embryo at day 25 of pregnancy was highest at the tip of the uterine horn and decreased towards the central portion of the uterus (Dziuk 1985). Possibly related to that, some authors reported that within uterine horns placental weights (from ~ day 30 of pregnancy onwards) and fetal weights (from ~ day 70 of pregnancy onwards) decrease from the ovarian end towards the cervical end of the uterine horn (Perry and Rowell 1969; Wise et al. 1997), whereas others found no evidence for a relation between location in the uterus and fetal weights (Van der Lende et al. 1990). Another factor that may possibly add additional within-litter variation in development could be a difference in uterine quality, such as distribution and density of uterine glands, among specific segments within one uterine horn, possibly related to the previous litter.

Mechanisms that may reduce within-litter variation during pregnancy

The existence of within-litter variation in development is inextricably connected with the reproductive strategy of the sow to overproduce, i.e. to produce a high number of piglets with low investment per piglet (Drake et al. 2008). This allows a high number of piglets to survive when conditions are optimal, thereby providing an extra benefit at minimal cost. As soon as conditions become suboptimal, the existence of within-litter variation in development provides a selective advantage, such that the larger piglets, which received more investment, are not harmed by the presence of the smaller 'surplus' piglets, which will die early and thus received little investment (Drake et al. 2008). In sows, therefore, there will always be within-litter variation in all stages of development, from oocytes to piglets born at term to piglets weaned. As soon as conditions become suboptimal, this within-litter variation ensures that selectively the less developed 'surplus' littermates are lost almost immediately.

In modern sows ~ 23 - 27 oocytes are released at ovulation (Gerritsen et al. 2008a, b; Foxcroft 2012; Hoving et al. 2012; Chapter 3 and 5), and only ~ 13 - 15 piglets are born at term (Chapter 7), and therefore still ~ 30 - 50% of the potential piglets are lost prenatally. Losses due to fertilization failure or lethal genetic defects that may occur before day 10

of pregnancy are generally low. Most prenatal losses occur during the peri-implantation period (~ day 12 - 16 of pregnancy) and during the fetal stage [from ~ day 30 of pregnancy onwards; Pope and First (1985); Geisert and Schmitt (2002); Foxcroft (2012)].

During early pregnancy, embryos become dependent on the continuously changing uterine environment. Asynchronous development of embryos and the uterine environment results in growth retardation or embryonic death. These changes in the uterine environment are triggered by the more developed embryos (Geisert and Schmitt 2002; Vallet et al. 2009). Selectively the lesser developed embryos will therefore be lost due to asynchrony with the uterine environment (Geisert and Schmitt 2002; Vallet et al. 2009) or because they do not develop fast enough to occupy sufficient uterine space for implantation. As a consequence, a higher uniformity of pre-implantation embryos likely results in a higher number of embryos surviving to ~ day 12 - 16 of pregnancy and thereby to implantation (until ~ day 25 - 30 of pregnancy) than a low uniformity of pre-implantation embryos. Additionally, high ovulation rates result in a higher number of embryos surviving to implantation (Van der Lende and Schoenmaker 1990; Vonnahme et al. 2002), which was also confirmed by the positive correlation between ovulation rate and the number of implantation sites as observed in this thesis ($r = 0.36$, $P = 0.02$; Figure 8.2a; adapted from Chapter 5). Both a higher number of ovulations (Van der Waaij et al. 2010) and a higher number of embryos surviving to implantation [i.e. number of implantation sites; Almeida et al. (2000); Vonnahme et al. (2002); Town et al. (2004)], however, result in smaller average placentas (length or weight), likely because the total available uterine space is divided over more embryos. In superovulated gilts, Van der Waaij et al. (2010) found that within-litter variation in placental weight at ~ day 40 of pregnancy also increased with increasing ovulation rate. Using the data of Chapter 5, negative relations between ovulation rate and mean placental length ($r = - 0.37$, $P = 0.01$) and dry weight ($r = - 0.37$, $P = 0.01$) and between total number of implantation sites and mean placental length ($r = - 0.35$, $P = 0.02$) and dry weight ($r = - 0.33$, $P = 0.03$) at day 42 of pregnancy were confirmed, as well as positive relations between ovulation rate and within-litter variation (CV) in placental length ($r = 0.48$, $P < 0.01$; Figure 8.2b) and dry weight ($r = 0.47$, $P < 0.01$) and between the total number of implantation sites and within-litter variation in placental length ($r = 0.47$, $P < 0.01$; Figure 8.2c) and dry weight ($r = 0.35$, $P = 0.02$). This likely reflects that a higher number of embryos that survive to implantation is associated with a higher number of relatively lesser developed embryos that survive, and thereby an increased within-litter variation in placental development. It is not clear whether an increased ovulation rate is

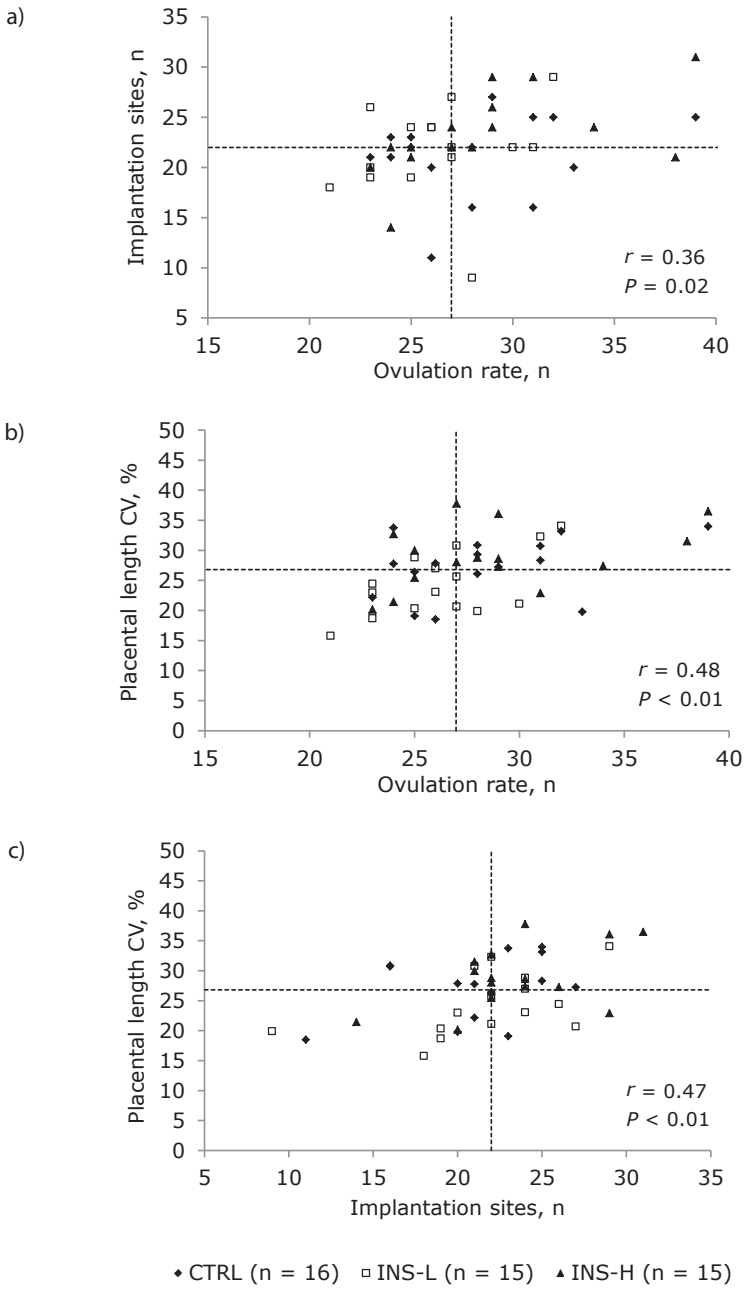


Figure 8.2 Relations between **a)** ovulation rate and the number of implantation sites; **b)** ovulation rate and within-litter CV of placental length (of vital fetuses); and **c)** total number of implantation sites and within-litter CV of placental length (of vital fetuses) at day 42 of pregnancy; dashed lines indicate the mean ovulation rate (27.0), mean number of implantation sites (22.1) and mean within-litter CV of placental length (26.8%); adapted from Chapter 5.

also associated with a higher number of poorer quality oocytes, and thereby an increased within-litter variation already in early embryos.

In modern sows, the number of ovulations, and thereby the number of embryos surviving to implantation, highly exceeds uterine capacity, and from ~ day 30 - 40 of pregnancy onwards uterine capacity becomes limiting for fetal survival (Vallet 2000; Vonnahme et al. 2002; Van der Waaij et al. 2010) and thereby determines the number of fetuses surviving to term. As a consequence, from ~ day 30 - 40 of pregnancy onwards the number of surviving fetuses is not related to ovulation rate anymore (Vonnahme et al. 2002; Van der Waaij et al. 2010; confirmed by data from Chapter 5 at day 42 of pregnancy: $r = 0.14$, $P = 0.34$), but e.g. associated with uterine horn length (Vonnahme et al. 2002). Besides uterine horn length (which determines the initial total uterine surface available for implantation), uterine capacity also involves factors as uterine blood flow and density of uterine glands. A lower uterine capacity probably results in a lower number of fetuses surviving to term. Fetuses with the smallest placentas will probably die first if the number of fetuses exceeds uterine capacity (Vallet 2000; Van der Waaij et al. 2010). Thus, independent of the within-litter variation in placental development at the start of the fetal stage (~ day 30 - 40 of pregnancy), a higher uterine capacity results in a higher number of fetuses surviving till term, and thereby also a higher survival of lesser developed fetuses. The more of these lesser developed fetuses survive, the higher the within-litter variation at term. This is confirmed by Van der Lende et al. (1990), who showed that litters (from ~ day 75 onwards till term) containing outliers with a low body weight, have a higher number of fetuses/piglets compared to litters with a normal distribution of fetal/piglet weights (in which the outliers with a low body weight probably died during earlier stages of pregnancy). A more limited uterine capacity, or a higher number of surviving fetuses, also results in a lower uterine blood flow per fetus, and thereby less nutrients extracted per fetus (Pere and Etienne 2000). This may also increase competition among littermates for blood flow and nutrients during pregnancy, and thereby possibly further increase within-litter variation in development, due to an unequal extraction of nutrients where the more developed fetuses with larger placentas will extract relatively more nutrients than the less developed fetuses.

In summary, within-litter variation at birth is the consequence of within-litter variation in early embryo development, which likely reflects variation in follicle and oocyte development. A higher within-litter variation in early embryo development will result in a higher within-litter variation at term. Furthermore, an uneven distribution of embryos

through the length of the uterine horns may add variation during early pregnancy, whereas increased competition among littermates for limited nutrients may further increase within-litter variation during later stages of pregnancy. On the other hand, selective losses of the lesser-developed peri-implantation embryos (~ day 12 - 16 of pregnancy) or the least developed fetuses (from ~ day 30 of pregnancy onwards) will remove part of the within-litter variation during pregnancy. Peri-implantation losses are directly related to the within-litter variation in early embryo development and ovulation rate, whereas fetal losses are determined by uterine capacity.

8.1.3 Effects of litter size and parity

Piglet birth weight and litter uniformity are strongly affected by litter size and parity (Chapter 6 and 7).

Litter size

Litter size effects on piglet birth weight and litter uniformity seem independent of sow parity and crossbreed, because interactions between parity and litter size or crossbreed and litter size were never significant. For each additional piglet born, mean piglet birth weight decreases with 40 - 41 g and CV of birth weight increases with 0.8% in sow populations with average litter sizes of 17.4 total born piglets (Chapter 6) and 13.5 total born piglets (Chapter 7; Table 8.2).

Table 8.2 Regression coefficients (βs) between the number of total born piglets and other litter characteristics at birth (based on total born)

	Total born, n	
Litter characteristic	Chapter 6[1]	Chapter 7[2]
Mean birth weight, g	- 40***	- 41***
CV of birth weight, %	0.76***	0.83***
Piglets < 800 g, %	1.5***	n.a.
Piglets < 1000 g, %	2.4***	1.9***

[1] Organic Topigs20 sows with 41 ± 4 day lactations and 17.4 ± 3.3 total born piglets (n = 115); relationships were corrected for the fixed effect of parity class (2, 3 + 4, ≥ 5) and random effect of sow (Chapter 6).
[2] Conventional Topigs20 and Topigs40 sows with 26 ± 4 day lactations and 13.5 ± 3.0 total born piglets (n = 2128); relationships were corrected for the fixed effects of parity class (1, 2, 3 + 4, ≥ 5) and crossbreed (Topigs20, Topigs40) and random effects of sow and farrowing batch; adapted from Chapter 7.
*** $P < 0.001$; n.a., not available.

Previous studies reported comparable effects of litter size on mean piglet birth weight (Van der Lende and De Jager 1991; Quiniou et al. 2002) and birth weight CV (Quiniou et al. 2002), in sow populations with average litter sizes up to 13 - 14 total born piglets. Table 8.3 shows effects of litter size on piglet birth weight and litter uniformity in recent studies with different parities and previous lactation lengths. The table shows that effects of litter size on piglet birth weight and litter uniformity appear consistent and linear over a broad range of litter sizes, which is further illustrated in Figure 6.2 (Chapter 6) for litter sizes ranging between 10 and 25 total born piglets.

High litter sizes result from high ovulation rates and thereby a high number of embryos surviving to implantation, and a high uterine capacity and thereby a high number of fetuses during pregnancy, which probably all contribute to the lower and less uniform birth weights within larger litters (see Paragraph 8.1.2). Another contributing factor to lower mean piglet birth weights with increasing litter sizes may be a generally shorter pregnancy length with increasing litter sizes (Rydhmer et al. 2008).

Parity

Piglet birth weight and litter uniformity are affected by parity (Milligan et al. 2002b; Damgaard et al. 2003; Quesnel et al. 2008). Results of this thesis confirm that piglets from first parity litters have lower mean birth weights than piglets from older sows, even after correction for litter size (Chapter 7). Mean birth weights peak in litters from 2nd to 4th parity sows and marginally decline in older sows (Milligan et al. 2002b; Damgaard et al. 2003; Quesnel et al. 2008), as confirmed in Chapter 7 even after corrections for litter size, but not in Chapter 6. Litter uniformity decreases with parity (Milligan et al. 2002b; Damgaard et al. 2003; Quesnel et al. 2008), and results of this thesis indicate that this parity effect remains significant after correction for litter size differences among parities (Chapter 6 and 7). Thus, both piglet birth weight and litter uniformity are affected by parity as such, in addition to litter size differences among parities.

Ovulation rates and thereby the number of embryos surviving to implantation generally increase with parity (Cox 1997; Town et al. 2005; Foxcroft et al. 2009) and also uterine capacity is higher in mature sows than in young sows, which explains the parity effect on litter size, and thereby part of the parity effects on piglet birth weight and litter uniformity (see Paragraph 8.1.2). Additionally, parity effects on piglet birth weight and litter uniformity may be related to a decline in follicle quality, uterine muscle tone and/or uterine quality with sow ageing. Reduced follicle development and oocyte quality with ageing is known to

Table 8.3 Litter characteristics at birth (based on total number born) for sow populations with different parities, litter sizes and lactation lengths

	Parity	Mean litter size, n	Previous lactation length, days		Litter size class (LSmeans)					*P*–value	
					≤ 9	10 to 11	12 to 13	14 to 15	≥ 16	Litter size	Parity[5]
Chapter 7[1] (n = 808)	3.2 ± 1.2	14.3 ± 3.0	26 ± 3	n	48	85	168	225	282		
				Litter size, n	7.7	10.5	12.6	14.5	17.5		
				Mean birth weight, kg	1.75	1.55	1.48	1.40	1.27	< 0.001	< 0.01
				CV of birth weight, %	15.6	19.1	20.4	22.0	24.3	< 0.001	< 0.001
Van den Brand et al. 2009[2] (n = 71)	5.6 ± 2.6	14.3 ± 2.9	26 ± 2	n	2	9	18	19	23		
				Litter size, n	8.5	10.1	12.4	14.6	17.5		
				Mean birth weight, kg	1.72	1.64	1.49	1.47	1.41	0.08	0.44
				CV of birth weight, %	17.0	20.3	21.3	24.1	27.3	0.01	0.67
Quesnel et al. 2008[3] (n = 1596)	3.0 ± 1.9	14.0 ± 3.7	n.a.	n	195	154	276	394	579		
				Litter size, n	7.1	10.6	12.6	14.5	17.7		
				Mean birth weight, kg	1.88	1.67	1.57	1.48	1.38	< 0.001	< 0.001
				CV of birth weight, %	15	18	21	22	24	< 0.001	< 0.001
Chapter 6[4] (n = 115)	4.7 ± 2.4	17.4 ± 3.4	41 ± 3	n	3	3	5	18	86		
				Litter size, n	7.7	10.7	12.6	14.7	18.8		
				Mean birth weight, kg	1.97	1.66	1.55	1.37	1.20	< 0.001	0.04
				CV of birth weight, %	9.6	15.2	19.3	20.6	24.3	< 0.001	< 0.01

[1] Adapted from Chapter 7; only including Topigs20 sows (Landrace x Large White).
[2] Adapted from Van den Brand et al. (2009); only including control Topigs20 sows (litter characteristics were influenced by a dietary treatment and therefore sows receiving this treatment were excluded).
[3] Adapted from Quesnel et al. (2008); Landrace x Large White sows.
[4] Adapted from Chapter 6; Topigs20 sows.
[5] For analysis divided into parity 2, 3 + 4, and ≥ 5 (no parity 1 sows were included), except for the study of Quesnel et al. (2008) in which parity 1 sows were also included and for analysis parity was divided into 1, 2, 3 + 4, 5 + 6, ≥ 7.
n.a., not available.

occur in humans (Broekmans et al. 2009), and possibly leads to increased developmental variation in oocyte quality and pre-implantation embryos, and thereby decreased litter uniformity in older sows. Reduced uterine muscle tone in older sows, generally associated with a hampered parturition process and thereby an increased number of stillborn piglets in older sows (Randall 1972; Zaleski and Hacker 1993), could possibly hamper an even spacing of embryos through the uterine horns in early pregnancy, and thereby add additional within-litter variation in older sows. Moreover, functional uterine capacity seems lower in older sows. Town et al. (2005) showed that despite an equal number of vital embryos at day 20 - 30 of pregnancy, sows of parity 5 and higher had a lower number of vital fetuses and a lower placental efficiency (calculated as the fetal:placental weight ratio) at day 50 - 90 of pregnancy compared to 3rd and 4th parity sows. More evidence for a decline in uterine quality with ageing comes from studies in mice (Finn 2001).

8.1.4 Summarizing

To conclude, piglet birth weight and litter uniformity are important factors for pre-weaning piglet survival, especially in large litters with high competition among littermates due to a relative decrease in the available number of functional teats. Both piglet birth weight and litter uniformity decrease with an increase in litter size. With increasing litter sizes in the future, therefore, piglet birth weight and litter uniformity will become more and more important. Results of this thesis indicate that even marginal improvements in piglet birth weight or litter uniformity can have substantial effects on pre-weaning piglet survival. Furthermore, although piglet birth weight and litter uniformity are strongly affected by sow factors as litter size and parity, still a large variation exists in piglet birth weight and litter uniformity among sows of similar parity and with equal litter sizes (as illustrated in Figure 6.2 for Topigs20 sows; Chapter 6). Together, this suggests that there are opportunities to improve piglet birth weight and litter uniformity, and thereby pre-weaning piglet survival, with a simultaneous increase in litter size.

8.2 EFFECTS OF PRE-MATING CONDITIONS RELATED TO SOW METABOLIC STATE

In this paragraph, effects of sow metabolic state during lactation and after weaning on subsequent piglet birth weight and litter uniformity, and on plasma insulin and IGF-1 levels as possible mediators of these effects, are discussed.

8.2.1 Effects on piglet birth weight and litter uniformity

Severe sow body condition loss, i.e. more than 10 - 12% weight loss, during a conventional 3 - 4 week lactation is known to suppress follicle development at weaning, and – as a consequence – suppresses subsequent reproductive performance [as reviewed by Quesnel (2009)]. Genetic selection for increased litter sizes and short weaning-to-estrus intervals has changed the response of sows to a catabolic state during (late) lactation from delayed estrus (Quesnel 2009) to reduced ovulation rates (Zak et al. 1997a; Van den Brand et al. 2000a) or embryo survival (Zak et al. 1997a; Van den Brand et al. 2000b; Vinsky et al. 2006; Hoving et al. 2012) and thereby reduced farrowing rates and litter sizes, and more recently towards reduced embryo development [as indicated by embryo weight or crown-rump length; Vinsky et al. (2006); Patterson et al. (2011); Hoving et al. (2012)], especially in sows weaned from their first litter. In Chapter 7, it is shown for the first time that, in sows with a short weaning-to-estrus interval (≤ 7 days), effects of severe body condition loss during a conventional 3 - 4 week lactation on subsequent reproduction can also be expressed in terms of reduced litter uniformity, independent of sow parity. For example, an increase in sow body weight loss from ≤ 3.5% (20% lowest class) to > 13% (20% highest class) resulted in an increase of 28 g in birth weight SD in the subsequent litter, and an increase in sow backfat loss during lactation from ≤ 2 (20% lowest class) mm to > 5 mm (20% highest class) resulted in an increase of 25 g in birth weight SD and 1.8% in birth weight CV in the subsequent litter. In contrast to negative effects of sow body condition loss during lactation on subsequent ovulation rates, embryo survival, farrowing rates and litter sizes, which generally only occur if body condition losses exceed a certain threshold (e.g. 10 - 12% of weight loss), the relation between sow body condition loss during lactation and subsequent litter uniformity seemed linear over the whole range of body weight loss from ≤ 3.5 to >13% and backfat loss from ≤ 2 to > 5 mm. These effects are likely related to compromised follicle development at weaning, and thereby possibly increased developmental variation within the pre-ovulatory follicle pool.

After weaning, sows quickly change towards an anabolic state. If the weaning-to-estrus interval or the interval from weaning to successful pregnancy is sufficiently prolonged, follicles and oocytes mostly develop during a period of an anabolic state, which may benefit their quality and thereby subsequent reproductive performance (see Chapter 1). In Chapter 7, the well-known beneficial effect of a prolonged weaning-to-pregnancy interval, i.e. a recovery period after weaning, on subsequent litter sizes [e.g. Clowes et al. (1994); Le Cozler et al. (1997); Van Leeuwen et al. (2011); Hoving (2012)] was confirmed in sows

with spontaneously prolonged weaning-to-pregnancy intervals (including sows with lactational estrus, silent estrus and repeat breeders). Sows with a weaning-to-pregnancy interval between 8 and 21 days or > 21 days (including repeat breeders) farrowed 1.2 and 0.7 piglets more, respectively, than sows with a regular short weaning-to-pregnancy interval of ≤ 7 days. Moreover, litter uniformity, corrected for these differences in litter sizes, was significantly higher in sows with a weaning-to-pregnancy interval of > 21 days (- 1.7% in birth weight CV), and numerically higher in sows with a weaning-to-pregnancy interval between 8 and 21 days (- 1.4% in birth weight CV) than in sows with a weaning-to-pregnancy interval of ≤ 7 days. This is the first study showing that a recovery period after weaning can be beneficial for subsequent litter uniformity, likely through restoration of follicle development after weaning.

If lactation is prolonged to 5 - 6 weeks, sows may already gradually change to an anabolic state during (late) lactation (as described in Chapter 1 and discussed in Chapter 6). In sows with prolonged lactations, therefore, follicles destined to ovulate after weaning mostly develop during a less severe catabolic state, or even an anabolic state, compared to sows with 3 - 4 week lactations. In Chapter 6, litter characteristics were studied in (organic) sows with 6-week lactation periods, and minimal body condition losses during their whole lactation (1.7 ± 0.7%). The high litter sizes of 17.4 ± 0.3 total born piglets found in Chapter 6 likely reflect an improved or restored follicle development at weaning. An improved development of follicles and oocytes at weaning is also expected to result in a higher litter uniformity at birth. A comparison of litter uniformity as found in Chapter 6 with other studies using conventional 3 - 4 week lactations is shown in Table 8.3. The large differences among studies in e.g. average litter size, parity and absolute mean piglet birth weights, however, do not allow conclusions about effects of a prolonged lactation on subsequent litter uniformity.

Mean piglet birth weights were not affected by sow body condition loss during previous lactation or by a prolonged weaning-to-pregnancy interval (Chapter 7). Although fetal growth is largely determined by placental size, which is largely fixed at day 35 of pregnancy and seems to be the consequence of within-litter variation in oocyte and early embryo development (see Chapter 1 and Paragraph 8.1), the absolute piglet weights at term may furthermore depend on other factors and mechanisms, such as sow genotype [e.g. Knol et al. (2010)], sow feed intake during pregnancy [e.g. Campos et al. (2012)], uterine blood flow and density of uterine glands (see Paragraph 8.1), and pregnancy length [e.g. Rydhmer et al. (2008)].

8.2.2 Insulin and IGF-1 as possible mediators

Effects of sow metabolic state on follicle and oocyte development, and thereby subsequent litter uniformity, are possibly mediated by insulin and/or IGF-1, because (i) insulin and IGF-1 are known to affect follicle and oocyte development, either indirectly via stimulation of gonadotropin release or directly at the ovarian level, where insulin and IGF-1 can act alone or by amplifying gonadotropin action (see Chapter 1); and (ii) plasma insulin and IGF-1 levels are closely related to sow metabolic state.

During a conventional 3 - 4 week lactation period, sows are in a catabolic state, related to high demands for milk production and a limited feed intake capacity (Quesnel and Prunier 1995). This catabolic state is accompanied by suppressed plasma insulin and IGF-1 levels (Rojkittikhun et al. 1993; Hoving et al. 2012). This was confirmed in Chapter 5, where plasma insulin and IGF-1 levels during late lactation were negatively related to sow body weight loss during lactation, and positively related to sow backfat thickness at weaning.

After weaning, sows quickly change towards an anabolic state associated with restoration of plasma insulin and IGF-1 levels (Quesnel et al. 1998; Van den Brand et al. 2001b; Hoving et al. 2012), which was also confirmed in Chapter 3 and 5. Insulin parameters after weaning were not related to pre-weaning insulin parameters (Chapter 5), nor to sow body weight and backfat loss during lactation (Chapter 3 and 5), indicating a quick restoration of insulin secretion after weaning. Plasma IGF-1 levels after weaning gradually increased (Chapter 3 and 5) and were strongly correlated to plasma IGF-1 levels during late lactation (Chapter 5), and thereby to sow body weight loss during lactation and backfat thickness at weaning (Chapter 5), indicating that restoration of IGF-1 secretion after weaning takes longer.

If lactation is prolonged from 3 - 4 weeks to 5 - 6 weeks, sows usually gradually recover from the catabolic state already during the last weeks of lactation (as described in Chapter 1). Rojkittikhun et al. (1993), for example, reported that sow body weight loss was lower during last two weeks of a 5-week lactation than during the first three weeks and Hultén et al. (2002) reported decreased body fat mobilisation (as indicated by plasma free fatty acid levels) during the last three weeks of a 5-week lactation period. Although not studied before, it can be speculated that plasma insulin and IGF-1 levels also gradually restore during the last weeks of a prolonged lactation of 5 - 6 weeks, if sow feed intake levels are sufficiently high.

8.2.3 Summarizing

For the first time it is shown that pre-mating conditions related to sow metabolic state during lactation or after weaning, known to affect follicle and oocyte development, can also affect subsequent litter uniformity. These effects on litter uniformity seem only marginal, probably because several mechanisms, such as the dynamics of prenatal losses, may modulate within-litter variation during pregnancy, and thereby influence the extent to which effects of developmental variation in the pre-ovulatory follicle pool are still visible in subsequent litter uniformity at term (see Paragraph 8.1). However, these marginal effects on litter uniformity may have substantial effects on pre-weaning piglet survival (see Paragraph 8.1).

These results confirm the hypothesis that litter uniformity at birth is already (partly) determined in the pre-mating period, likely related to (insufficient) restoration of follicle development and uniformity, and possibly mediated by insulin and/or IGF-1.

8.3 EFFECTS OF INSULIN-STIMULATING DIETS DURING THE WEANING-TO-ESTRUS INTERVAL

As a first step to further test the hypothesis that pre-mating conditions, and especially pre-mating plasma insulin and IGF-1 levels, can affect follicle and oocyte development and their uniformity and thereby subsequent litter uniformity at different stages of pregnancy, a series of physiological experiments was conducted with insulin-stimulating diets during the weaning-to-estrus interval in multiparous sows. Multiparous sows were used in these experiments, because these sows generally have lower litter uniformity compared to young sows (as described in Chapter 1 and discussed in Paragraph 8.1). In this paragraph, effects of insulin-stimulating diets on plasma insulin and IGF-1 levels, and on follicle development and subsequent litter uniformity are discussed. Thereafter, possible factors modulating effects of insulin-stimulating diets during the weaning-to-estrus interval, and possible effects of insulin-stimulating diets during lactation, on litter uniformity are discussed.

8.3.1 Effects on plasma insulin and IGF-1 levels

Insulin

Plasma insulin levels can be easily enhanced with dietary sugars as dextrose and sucrose, which result in fast and high insulin peaks directly after feeding (Chapter 2 and 5). The

inclusion of 150 g/day dextrose or 150 g/day sucrose only resulted in numerically increased insulin responses compared to an isocaloric control diet (Chapter 2). For a more effective insulin stimulation, dextrose or sucrose can better be included at higher levels, or in combination with additional starch (slower available glucose). In Chapter 5 it is shown that the combination of dextrose plus starch results in both fast and high insulin peaks directly after feeding (within 1 hour after feeding), as well as long-term enhanced insulin levels (~ 4 hours after feeding), in a dose-dependent manner.

Vestergaard (1997) showed in gilts that a diet containing large amounts of sugarbeet pulp (500 g/kg) resulted in sustained higher insulin levels from 3 - 4 hours after feeding compared to a standard diet, probably through proprionate, one of its fermentation end products which can subsequently be used for glucose production. Therefore, it was hypothesized that a diet supplemented with both sugars and fermentable carbohydrate sources enhances both insulin peak levels and peak duration. In Chapter 2, however, large amounts of sugarbeet pulp (400 g/kg; 1200 g/day) did not result in the expected sustained enhanced insulin levels up to 6 hours after feeding in multiparous sows. De Leeuw et al. (2004) and Serena et al. (2009) also failed to show sustained enhanced insulin levels up to 10 - 11 hours after feeding comparable large amounts of sugarbeet pulp in first parity sows. Moreover, the combination of both sugar (such as dextrose) and sugarbeet pulp was not beneficial for insulin stimulation, because it did not result in fast and high insulin peaks directly after feeding either. This is likely related to the physicochemical properties of sugarbeet pulp in the gastrointestinal tract (see Chapter 2).

Besides sugarbeet pulp, lactose was used as a fermentable carbohydrate source in Chapter 2. Adult sows have a limited lactase activity in the small intestine and consequently significant amounts of the ingested lactose will flow into the large intestine to be fermented. However, lactose did not result in sustained enhanced insulin levels after feeding either (see Chapter 2).

Feeding twice a day results in high insulin peaks twice a day, but also relatively long periods of low (basal) insulin levels in between feedings. In an attempt to keep insulin levels enhanced and more constant for a prolonged period of the day, the feeding frequency was increased to six times per day at a similar daily feed allowance in Chapter 3 and 4. However, frequent feeding of an insulin-stimulating diet (with dextrose plus lactose, both 150 g/day) did not result in a more sustained period of increased insulin levels, likely related to a more than proportional reduction in postprandial insulin response with smaller meal

sizes at a higher feeding frequency. To further test this hypothesis, sows were fed a 0.5 kg portion of the dextrose plus lactose diet (both 50 g/kg) and the postprandial insulin response was compared to their postprandial response after the 1.5 kg portion of the same dextrose plus lactose diet (as fed in Chapter 2), both determined after an overnight fast of 16 hours (Figure 8.3; adapted from Chapter 2; n = 9 sows in cross-over design). The postprandial insulin response (area under the curve) was 869 ± 187 μU/3.8 hours after the 0.5 kg portion (and insulin levels had already reached basal levels within 3.8 hours), which was indeed remarkably lower than the proportional one third of the postprandial insulin response after the 1.5 kg feed portion (the insulin response after the 1.5 kg portion was 4328 ± 509 μU/12 hours, assuming that insulin levels would gradually decrease to basal levels at 12 hours after feeding; based on results of Chapter 3). This confirms that the insulin response remains relatively lower after feeding smaller meal sizes. Factors contributing to this more than proportional lower insulin secretion with smaller meal sizes may be a lower secretion of insulin-stimulating incretins in response to a lower amount of ingested nutrients, which is for example shown in human (Jenkins et al. 1990, 1992), either or not in combination with a reduced rate of gastric emptying. Moreover in Chapter 3, short-term increases in pre-prandial free fatty acid levels related to the long (12 hours) fasting interval between feedings may have been an additional factor stimulating

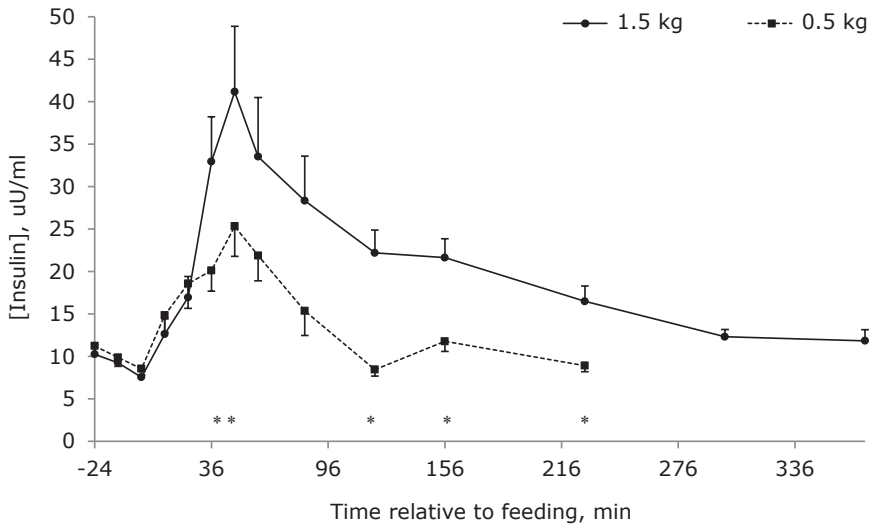


Figure 8.3 Insulin profiles (means ± SE) around 0800 hour feeding (after a 16 hour-fasting period) for a 1.5 kg portion and a 0.5 kg portion of the dextrose plus lactose diet (both 50 g/kg); n = 9 sows in a cross-over design; * = 1.5 vs. 0.5 kg, $P \leq 0.05$; adapted from Chapter 2.

glucose-stimulated insulin secretion in the control sows (see Chapter 5), but probably not in the treatment sows with only 4 hour fasting intervals. Although two completely different insulin secretion patterns were created by modulating both diet composition and feeding frequency in Chapter 3 (see also Figure 3.1), increasing the feeding frequency without increasing total daily feed intake is clearly not a proper way to stimulate insulin secretion and to keep insulin enhanced and more constant for a prolonged period of the day.

Insulin-like growth factor-1

Although insulin levels can be easily modulated with insulin-stimulating feed components and feeding frequency, modulation of plasma IGF-1 levels by insulin-stimulating dietary treatments seems limited, both in anabolic non-pregnant sows recovered from lactation (at > 30 days after weaning; Chapter 2) and in newly weaned sows (Chapter 3 and 5). In anabolic sows, insulin is probably not limiting for hepatic growth hormone (GH) binding and thereby GH-stimulated IGF-1 production, but in catabolic sows, insulin can improve hepatic GH binding and thereby stimulate IGF-1 production (see Chapter 1 and 2). After weaning, sows switch from a catabolic state to an anabolic state (see Paragraph 8.2). Independent of insulin-stimulating diets during the weaning-to-estrus interval, plasma IGF-1 levels show already a large increase during the first days after weaning (Chapter 3 and 5; Van den Brand et al. 2001b). If insulin would be limiting for hepatic GH binding and thereby GH-stimulated IGF-1 production during these first days after weaning, an effect of the insulin-stimulating diets on plasma IGF-1 levels would be expected to occur within one or two days after weaning, which was not the case (Chapter 3 and 5).

At day 4 and 5 after weaning, however, plasma IGF-1 levels were higher in sows with a high (voluntary) feed intake (≥ 75%) than in sows with a low (voluntary) feed intake (< 75%) during the weaning-to-estrus interval (Chapter 3), and plasma IGF-1 levels at day 3 after weaning were numerically increased by insulin-stimulating diets (dextrose plus starch in Chapter 5; plasma IGF-1 levels at day 4 and 5 were not measured). Moreover, plasma IGF-1 levels around weaning (the day before, at, and after weaning) were positively correlated to insulin parameters at the day before weaning (Chapter 5), whereas plasma IGF-1 levels during days 2 - 5 after weaning were positively related to insulin parameters at days 2 and 3 after weaning (Chapter 3 and 5). This could indicate that insulin stimulation through either a high feed intake or through insulin-stimulating diets during the weaning-to-estrus interval may result in higher IGF-1 levels from 4 - 5 days after weaning onwards. Due to this possible latency in IGF-1 response to insulin-stimulating diets after weaning,

however, the weaning-to-estrus interval may be too short for an effective stimulation of IGF-1 secretion by insulin-stimulating dietary treatments. Because plasma IGF-1 levels during the first days after weaning were strongly related to plasma IGF-1 levels during late lactation (see Chapter 5 and Paragraph 8.2), and thereby to sow body condition loss during lactation (Chapter 5), focus should be on increased IGF-1 levels during late lactation to stimulate IGF-1 levels during the first days after weaning. In primiparous sows, both an increased feeding level and insulin-stimulating diets were effective stimulators of plasma IGF-1 levels during lactation (Van den Brand et al. 2001b).

In summary, postprandial insulin responses (both fast peaks and long-term insulin level) were successfully stimulated during the weaning-to-estrus interval by feeding insulin-stimulating diets supplemented with different levels of dextrose plus starch (both at 375 g/day or both at 172 g/day) twice a day, and thereby resulted in large contrasts in insulin responses among sows in Chapter 5. In the experiment of Chapter 3 and 4, total insulin secretion and absolute insulin levels were not successfully stimulated, although contrasts were created in the insulin secretion pattern during the weaning-to-estrus interval by feeding an insulin-stimulating diet (dextrose plus lactose, both 150 g/day) at 4 hour-intervals compared to an isocaloric control diet at 12 hour-intervals. Unfortunately, plasma IGF-1 levels were not significantly affected by the dietary treatments until 3 (Chapter 5) or 5 (Chapter 3) days after weaning, and thus treatment effects on follicle development and subsequent litter uniformity may reflect effects of plasma insulin levels rather than plasma IGF-1 levels. Moreover, it should be realized that in the experiment in Chapter 3 (and 4) absolute insulin levels and IGF-1 levels (from ~ day 3 - 4 after weaning) were very variable among sows, but this was due to a high variation in voluntary feed intake, and thereby a high proportion of sows with a low feed intake, during the weaning-to-estrus interval.

8.3.2 Effects on follicle development and consequences for litter uniformity

The insulin-stimulating diets, or dietary treatments, during the weaning-to-estrus interval did not have the expected effects on follicle development and subsequent litter uniformity in the three experiments described in this thesis (Chapter 3 and 4, 5 and 6).

In Chapter 3 and 4, the dextrose plus lactose diet fed at 4 hour-intervals during the weaning-to-estrus interval resulted in a lower pre-ovulatory LH surge, smaller and less uniform follicles at day 4 after weaning and subsequently smaller corpora lutea and numerically less developed

embryos at day 10 of pregnancy compared to the control diet fed at 12 hour-intervals. This may reflect an effect of the pattern of insulin secretion during the day (six short insulin peaks per day vs. two sustained insulin peaks per day) rather than an effect of total insulin secretion or absolute insulin levels. Because diurnal variation exists in insulin responses (see Chapter 5), however, the total daily insulin secretion in Chapter 3 and 4 can not be extrapolated from the postprandial insulin responses which were only measured at the 0800 hour feedings. Besides the insulin pattern, the possible role of other factors related to and/or modulated by the higher feeding frequency, such as changes in other metabolites/metabolic hormones (e.g. leptin and free fatty acids), the existence or disruption of circadian rhythms in sleep-wake cycles and/or the gastrointestinal tract (Scheving 2000; Konturek et al. 2011), and stress, cannot be excluded. Independent of treatment, however, insulin levels during the first three days after weaning were positively related to LH secretion, follicle diameter and subsequent progesterone levels during the first 10 days of pregnancy, and plasma insulin levels tended to be positively related to embryo development (diameter and DNA content; but not uniformity) at day 10 of pregnancy in Chapter 3 and 4.

The extreme insulin-stimulating diets fed twice daily during the weaning-to-estrus interval in Chapter 5 did not improve follicle development (average diameter of five largest follicles on one ovary) or subsequent development and uniformity of fetuses and placentas at day 42 of pregnancy. Plasma insulin and IGF-1 levels during the first three days after weaning were neither related to follicle development or subsequent fetal and placental development and uniformity, although a positive relation between the postprandial insulin response (as indicated by maximal insulin level after feeding) at day 2.5 after weaning and progesterone level at day 42 of pregnancy was also found. Because there were no differences in number and development of fetuses and placentas at day 42, and uterine capacity was probably not influenced by the dietary treatments, it is not likely that these diets would have resulted in improved litter uniformity at later stages of pregnancy and at term.

In Chapter 6, the insulin-stimulating diets during only the weaning-to-estrus interval, or during the last two weeks of a 6-week lactation period plus the weaning-to-estrus interval, did not result in improved piglet birth weights or uniformity of the subsequent litter.

In summary, uniformity of embryos, fetuses or placentas, or piglets was not influenced by the insulin-stimulating diets, nor related to plasma insulin and IGF-1 levels, during the weaning-to-estrus interval. Thus, the physiological experiments in this thesis do not confirm that nutritionally increased plasma insulin and IGF-1 levels during only the

weaning-to-estrus interval can improve follicle development and uniformity, and thereby subsequent litter uniformity in multiparous sows. Possible explanations why the hypothesis of this thesis was not confirmed are discussed in the following paragraph.

8.3.3 Possible factors modulating effects of insulin-stimulating diets during the weaning-to-estrus interval

Table 8.4 compares diet and sow characteristics among studies regarding insulin-stimulating diets before mating. Among studies, differences exist in for example the period during which insulin-stimulating diets were fed, the evaluation moment of litter uniformity, and sow characteristics as parity and body condition loss during lactation (Table 8.4). Two studies (Van den Brand et al. 2006, 2009) found positive effects of pre-mating insulin-stimulating diets on litter uniformity, whereas three studies did not (Chapter 3 and 4, Chapter 5, Chapter 6; Table 8.4). Inconsistent effects of insulin-stimulating diets during the weaning-to-estrus interval on litter uniformity may be related to the prenatal assessment of litter uniformity, IGF-1 status after weaning, follicle development at weaning and/or the short weaning-to-estrus interval and ongoing genetic improvements in modern sows, which will be discussed below.

Prenatal assessment of litter uniformity

In the physiological experiments in this thesis litter uniformity was assessed prenatally by: (i) development and uniformity of follicles at day 4 after weaning (Chapter 3 and 5); (ii) development and uniformity of pre-implantation embryos at day 10 of pregnancy (Chapter 4); and (iii) development and uniformity of fetuses and placentas at day 42 of pregnancy (Chapter 5).

Follicle development and uniformity was assessed by measuring the diameter of all follicles ≥ 3 mm, assumed to represent the pre-ovulatory follicle pool (Chapter 3) or by measuring the five largest follicles at one ovary (Chapter 3 and 5) at day 4 after weaning, using ultrasound. Circumstantial evidence exists that follicular development (assessed based on steroid content and moment of ovulation) and oocyte maturation are skewed, with a majority being further developed than a lesser developed minority, and that this developmental variation in pre-ovulatory follicles is reflected in subsequent within-litter embryo variation until ~ day 12 of pregnancy (Pope et al. 1988, 1990; Xie et al. 1990a, b, c; also see Chapter 1). Follicle diameter is generally positively correlated to follicular fluid

Table 8.4 Comparison of dietary treatments and sow characteristics among studies regarding effects of pre-mating insulin-stimulating diets on litter uniformity (means ± SE)

	Chapter 3 + 4	Chapter 5	Chapter 6	Van den Brand et al. (2006)	Van den Brand et al. (2009)
Number of sows[1]	32	54	115	176	134
Dietary treatments					
Insulin-stimulating feed components used	Dextrose + lactose (both 150 g/day)	Dextrose + starch (both 375 g/day or both 172 g/day)	Sucrose + lactose (both 150 g/day)	Dextrose (150 g/day)	Dextrose plus lactose (both 150 g/day)
Period	WEI	WEI	Last 2 wks lactation + WEI, or only WEI	WEI	Lactation + WEI
Isocalorically exchanged or topdressing	Isocalorically exchanged	Isocalorically exchanged	Lactation: isocalorically exchanged WEI: topdressing	Topdressing	Lactation: isocalorically exchanged WEI: topdressing
Feeding frequency	6 x / day[2] (CON 2 x /day)	2 x / day	Lactation: 2 x / day WEI: 1 x / day	2 x / day	2 x / day
Results					
Evaluation moment	Day 10 of pregnancy	Day 42 of pregnancy	At term	At term	At term
Effects on litter uniformity	None	None	None	CV: – 3.7% (21.2 vs. 17.5%; $P = 0.03$)[3]	CV: – 3.2% (23.7 vs. 20.5%; $P = 0.04$)
Effects on average litter development	Mean embryo diameter: - 0.7 mm (7.1 vs. 6.4 mm; $P = 0.07$)[4]	None	None	None	Mean birth weight: + 89 g (1465 vs. 1554 g; $P = 0.05$)

	Chapter 3 + 4	Chapter 5	Chapter 6	Van den Brand et al. (2006)	Van den Brand et al. (2009)
Sow characteristics					
Parity (after treatment)	5.9 ± 0.3	5.7 ± 0.1	4.6 ± 0.2	3.0 ± 0.2	5.4 ± 0.2
Previous lactation					
Lactation length, days	25 ± 1	26 ± 1	41 ± 4	26 ± 1	27 ± 1
Body weight loss, %	12.0 ± 0.5	8.8 ± 0.6	1.7 ± 0.7[5]	8.6 ± 0.6[5]	4.1 ± 0.5[5]
Backfat loss, mm	5.1 ± 0.3	4.6 ± 0.3	n.a.	5.1 ± 0.2	4.4 ± 0.2
Follicle size at weaning, mm	3.4 ± 0.1	4.0 ± 0.1[6]	n.a.	n.a.	n.a.
Weaning-to-estrus interval					
Dry matter intake until estrus, %	69 ± 6	99 ± 4	n.a.	n.a.	n.a.
Follicle size at day 4, mm	6.8 ± 0.2	6.4 ± 0.1	n.a.	n.a.	n.a.
Weaning-to-estrus interval, h	96 ± 2	102 ± 3	~ 101[7]	105 ± 1	112 ± 1
Ovulation rate, n	23.7 ± 0.5	27.0 ± 0.6	n.a.	n.a.	n.a.
Pregnancy					
Vital embryos/fetuses, n	21.1 ± 0.7	18.6 ± 0.6	n.a.	n.a.	n.a.
Litter size at term, n	n.a.	n.a.	17.4 ± 0.3	13.8 ± 0.3	14.3 ± 0.3

[1] In all studies, the same genotype (Topigs20) was used.
[2] The modulation of both diet composition and feeding frequency resulted in contrasts in the insulin secretion patterns during the weaning-to-estrus interval (six short peaks per day vs. two sustained peaks per day), but absolute insulin secretion and insulin levels were not successfully stimulated.
[3] Based on live born piglets only (because stillborn piglets were not individually weighed).
[4] Additionally, positive relations were found between insulin levels during the first three days after weaning and progesterone levels during the first 10 days of pregnancy, and plasma insulin levels tended to be positively related to embryo development (diameter and DNA content) at day 10 of pregnancy.
[5] Sow body weight at farrowing was estimated by subtracting the weight of the litter at birth (live born and stillborn), estimated placenta and amnion weight [216 g/piglet; based on Van Rens and Van der Lende (2002)] and estimated fetal growth during the days between weighing of the sow and farrowing [40 g/fetus/day; based on McPherson et al. (2004)] from sow body weight at entering the farrowing stable.
[6] Determined at ~ 16 hours after weaning.
[7] 4.2 ± 0.1 days.
WEI, weaning-to-estrus interval; n.a., not available.

estrogen content [reviewed by Foxcroft and Hunter (1985); Hunter and Wiesak (1990)], and both follicle diameter and estrogen secretion are generally positively correlated to stage of oocyte maturation (Hunter and Wiesak 1990). However, still considerable variation in follicular fluid estrogen content and oocyte maturation can exist among follicles of identical size (Foxcroft and Hunter 1985; Hunter and Wiesak 1990). It remains debatable, therefore, whether follicle diameter at day 4 after weaning is an accurate indicator for oocyte quality, and thereby subsequent embryo uniformity. However, measurement of follicle diameter by ultrasound is the only non-invasive way to assess follicle development without interfering with processes as ovulation, fertilization and early embryo development.

Development and uniformity of pre-implantation embryos was assessed based on embryoblast diameter, conceptus diameter, conceptus protein content and conceptus DNA content at day 10 of pregnancy. Day 10 of pregnancy was chosen as the first evaluation moment to get a complete overview of the total within-litter variation in embryo development prior to implantation; after day 10 of pregnancy part of the within-litter variation is lost as a consequence of prenatal losses during implantation (see Paragraph 8.1). Indeed in Chapter 4, average embryo recovery rate (as % of ovulation rate) at day 10 of pregnancy was 89 ± 2%. At this early stage of pregnancy, the conceptus consists of the embryoblast, which will develop into the fetus, and extra-embryonic membranes, which will develop into to the placenta. Until ~ day 10 of pregnancy, pig conceptus remain spherical. Once reaching 9 - 10 mm in diameter, the conceptuses very rapidly (~ 30 - 45 mm/hour) undergo a morphological transformation to tubular (10 - 40 mm) and finally thin filamentous thread-like shapes (> 100 mm in length) in less than 3 - 4 hours (Pope et al. 1990; Geisert and Yelich 1997). Transformation from spherical to filamentous shapes occurs through massive cellular remodeling rather than cell division (Geisert and Yelich 1997). Due to this very rapid development of conceptuses at this stage, conceptus development ~ day 10 is a highly variable trait, both among sows and within sows. It remains debatable, therefore, whether and which conceptus characteristics (embryoblast diameter, conceptus diameter, conceptus protein content, conceptus DNA content) at day 10 of pregnancy are appropriate indicators for early embryo and placental development and thereby reliable predictors for litter uniformity at birth, especially in litters containing tubular or filamentous conceptuses. In Chapter 4, 81% (22/27) of the litters contained only spherical conceptuses, whereas only 3 litters contained already some tubular conceptuses and only 2 litters contained filamentous conceptuses. In most of these litters, therefore, these conceptus characteristics seem to be appropriate for assessment of litter uniformity.

At day 42 of pregnancy (Chapter 5), placental development characteristics probably are more reliable predictors for subsequent fetal development and thereby piglet uniformity at birth than fetal development characteristics, because placental development is not limiting for fetal growth until ~ day 50 - 70 of pregnancy onwards (Van der Lende 1989; Foxcroft et al. 2009). Although uterine blood flow and placental vascularity were not measured in Chapter 5, measures of placental length, dry weight and implantation length together should have been appropriate to reveal effects of insulin-stimulating diets on litter uniformity, in case they existed.

Role of IGF-1 status after weaning

Insulin-stimulating diets during the weaning-to-estrus interval did not effectively enhance plasma IGF-1 levels during the first three days after weaning (Chapter 3 and 5). The main role of insulin is to provide cells with glucose (fuel), whereas the main role of IGF-1 is to stimulate cellular mitosis and differentiation (growth factor; as described in Chapter 1), and likely both fuel and growth factors are needed to stimulate follicle development, i.e. follicle cell growth and differentiation. It can be speculated, thus, that both high plasma insulin levels and high plasma IGF-1 levels are required for a beneficial effect of insulin-stimulating diets on follicle development and thereby subsequent litter uniformity. In Chapter 3 and 4, and in Chapter 5, IGF-1 levels after weaning were suppressed due to the catabolic state during lactation (see also Paragraph 8.2) and therefore may have been insufficient for the insulin-stimulating diets to exert positive effects on follicle development and subsequent litter uniformity (Table 8.4). In the study of Van den Brand et al. (2006), the insulin-stimulating diet was also fed only during the weaning-to-estrus interval, but average parity was considerably lower compared to the other studies (Table 8.4), because mainly sows after weaning their 1st (42%) or 2nd litter (31%) were used. Clowes et al. (1994) reported higher absolute plasma IGF-1 levels at first estrus after weaning in first and second parity sows than in higher parity sows (97 ± 11 vs. 70 ± 9 ng/ml; $P = 0.09$), which was also confirmed by data of our lab where plasma IGF-1 levels were measured at three days after weaning in primiparous (205 ± 19 ng/ml) and multiparous sows (154 ±14 ng/ml; NM Soede, unpublished results). Moreover, post-weaning plasma IGF-1 increases are generally higher in young sows than in older sows (Chapter 3 and 5; Clowes et al. 1994; Van den Brand et al. 2001b; Langendijk et al. 2008). These higher IGF-1 levels after weaning in younger sows are probably related to their higher requirements for lean tissue growth compared to higher parity sows. The use of younger sows with generally higher IGF-1 levels after

weaning may explain why insulin-stimulating diets after weaning had positive effects on litter uniformity in the study of Van den Brand et al. (2006), in contrast to the studies in Chapter 3 and 4, and in Chapter 5 in which mainly sows of parity ≥ 5 were used (Table 8.4). Because plasma IGF-1 levels likely restored during the last two weeks of the 6-week lactation in the multiparous sows of Chapter 6, a positive effect of the insulin-stimulating diets during the weaning-to-estrus interval on litter uniformity could be expected, but was not found (Table 8.4). This may, however, be related to their improved or restored follicle development at weaning, as indicated by their high subsequent litter sizes (17.4 ± 0.3 total born piglets; see Paragraph 8.2), which can probably not be further improved by insulin-stimulating diets.

Role of follicle development at weaning

Another explanation for the inconsistent effect of insulin-stimulating diets during the weaning-to-estrus interval on litter uniformity could be related to the degree to which follicle development was compromised at weaning, which in turn is related to sow body condition loss during lactation. Severe sow body condition loss during lactation is known to suppress follicle development at weaning, but subsequent reproductive responses to a catabolic state during lactation are very variable among sows and sow populations (see Paragraph 8.2). It can be speculated that the level of developmental variation within the follicle pool at weaning may determine how sows will respond to insulin-stimulating diets after weaning, and that insufficient restoration of follicle development at weaning may increase developmental variation within the follicle pool (as indicated by the relation between sow body condition loss during lactation and subsequent litter uniformity; see Chapter 7 and Paragraph 8.2). If developmental variation in the follicle pool becomes too large, it may either reduce ovulation rates, because fewer follicles acquired sufficient LH receptors on their granulosa cells to be able to respond to the shift in LH release pattern leading to ovulation (see Chapter 1), or it may reduce embryo survival at the peri-implantation period related to a larger proportion of embryos dying due to asynchrony with the uterine environment (see Paragraph 8.1); in this case insulin-stimulating diets after weaning likely will restore ovulation rates and embryo survival. This may occur for example in young sows with high body condition losses during lactation, because young sows are most sensitive to adverse effects of body condition loss (Thaker and Bilkei 2005; Quesnel 2009). If developmental variation in the follicle pool is only low to moderately increased, ovulation rates and embryo survival may be less affected, but effects of higher developmental variation in follicle development are likely expressed in

terms of reduced embryo development and litter uniformity at the post-implantation period, and consequently reduced litter uniformity at term, related to uterine capacity (see Paragraph 8.1); in this case insulin-stimulating diets after weaning more likely improve litter uniformity. This may occur for example in higher parity sows with high body condition losses during lactation or in young sows with moderate body condition losses during lactation. Sows with low body condition loss during lactation and sows with prolonged lactations probably do not have a compromised follicle pool at weaning, and therefore it is not likely that insulin-stimulating diets after weaning further improve follicle development. This may explain why in Chapter 6 no effects of the insulin-stimulating diets on subsequent litter uniformity were found. The multiparous sows in Chapter 5 had a low to moderate body weight loss during lactation of 8.8 ± 0.6%, had relatively large follicles of 4.0 ± 0.1 mm at 16 hours after weaning and 27.0 ± 0.6 subsequent ovulations (Table 8.4). This may indicate that follicle development was insufficiently compromised in these sows for the insulin-stimulating diets to be effective. The multiparous sows in Chapter 3 and 4 had moderate to high body weight loss during lactation of 12.0 ± 0.5%, smaller follicles at weaning (3.4 ± 0.1 mm), and lower ovulation rates (23.7 ± 0.5; Table 8.4), which may indicate that follicle development was moderately compromised in these sows. This may explain why in Chapter 3 and 4 insulin levels during the first three days after weaning were positively related to progesterone levels and embryo development during the first 10 days of pregnancy. Uniformity of embryos, however, was not related to insulin, but this may be related to the relatively low number of pregnant animals or the use of inappropriate early prenatal predictors for litter uniformity at term. In the study of Van den Brand et al. (2006), the – much younger – sows lost 8.6 ± 0.6% of body weight during lactation (Table 8.4). More specifically, sows that weaned their 1st and 2nd litter (parity 2 and 3 sows after treatment) only had moderate body weight losses of 7.6 ± 0.7%, whereas higher parity sows had high body weight losses of 11.0 ± 1.0%. This may indicate that both the young sows and the higher parity sows had a moderately compromised follicle development at weaning, which may explain the positive effects of the insulin-stimulating diets after weaning found on litter uniformity in that study.

Role of short weaning-to-estrus intervals and ongoing genetic improvements

Strong genetic selection of sows on the interval to estrus has resulted in short weaning-to-estrus intervals of only 4 - 5 days in modern sows (Table 8.4). Positive effects of insulin-stimulating diets or daily insulin injections during only the weaning-to-estrus interval of first parity weaned sows on follicle development, interval to estrus, ovulation rate, and

subsequent farrowing rates and litter sizes have been reported before (King and Williams 1984; Ramirez et al. 1997; Whitley et al. 1998a, b, c and 2002; Van den Brand et al. 2001a), but these sows generally had longer weaning-to-estrus intervals. In multiparous sows with short weaning-to-estrus intervals (< 5 days), generally no effects of insulin-stimulating diets or daily insulin administration after weaning on interval to estrus, farrowing rates and litter sizes are found (Kirkwood and Thacker 1991; Whitley et al. 2002). It is possible that in modern sows, the weaning-to-estrus interval has become too short to influence follicle development and uniformity and thereby subsequent litter uniformity. This would mean that insulin-stimulating diets should be given also during lactation to have significant effects on follicle development and uniformity (which will be further discussed in Paragraph 8.3.4). However, this does not explain why Van den Brand et al. (2006) did find effects of insulin-stimulating diets during the weaning-to-estrus interval on subsequent litter uniformity in sows with a weaning-to-estrus interval of less than 5 days (Table 8.4).

Genetic selection not only has resulted in sows with short weaning-to-estrus intervals and high litter sizes; both the interval to estrus and litter sizes also have become more resistant to the negative effects of severe body condition loss during lactation in modern sows (see Paragraph 8.2). From ~ 2002 onwards, piglet survival and litter uniformity are also included in genetic selection programs (EF Knol, TOPIGS Research Center IPG, The Netherlands; personal communication). It can be speculated, therefore, that the inclusion of both piglet survival and litter uniformity in genetic selection programs will result in sows that farrow litters with on average higher litter uniformity, and ultimately sows may become less sensitive to the negative consequences of severe body condition loss during lactation on subsequent litter uniformity. In Chapter 7 it appeared that effects of severe body condition loss during lactation on subsequent litter uniformity were substantially more pronounced in Topigs20 sows than in Topigs40 sows. This may indicate that negative consequences of severe body condition loss during lactation on subsequent litter uniformity may differ between crossbreed lines and may also be responsive to genetic selection. All studies regarding effects of insulin-stimulating diets during the weaning-to-estrus interval on litter uniformity (Table 8.4) used the same crossbreed line, which is Topigs20, but studies were performed in 2003 (Van den Brand et al. 2006), 2008/2009 (Chapter 6), 2009 (Chapter 3 and 4), and 2011 (Chapter 5). Ongoing genetic progress, thus, may be another factor influencing effects of insulin-stimulating diets during the weaning-to-estrus interval on litter uniformity, and may be involved in the less pronounced effects found on litter uniformity in the most recent studies (Table 8.4).

In summary, the physiological experiments in this thesis do not confirm that insulin-stimulating diets during the weaning-to-estrus interval can improve follicle development and subsequent litter uniformity in multiparous sows. This may be related to the (early) prenatal evaluation of litter uniformity in these experiments and/or the short weaning-to-estrus intervals and ongoing genetic improvements in modern sows. Furthermore, it is hypothesized that insulin-stimulating diets during the weaning-to-estrus interval may only be beneficial for follicle development and subsequent litter uniformity in combination with:

(i) sufficiently high plasma IGF-1 levels after weaning. Plasma IGF-1 levels after weaning are strongly related to sow body condition loss during lactation and insulin-stimulating diets during the weaning-to-estrus interval do not effectively stimulate IGF-1 levels after weaning. Plasma IGF-1 levels after weaning in multiparous sows may, therefore, be insufficient for the insulin-stimulating diets during only the weaning-to-estrus interval to exert positive effects on follicle development and subsequent litter uniformity. In younger sows, plasma IGF-1 levels after weaning are higher and probably sufficient for insulin-stimulating diets to improve follicle development; and/or

(ii) low to moderately compromised follicle development at weaning. If follicle development is highly compromised at weaning, insulin-stimulating diets after weaning are more likely to restore ovulation rates and embryo survival. If follicle development is low to moderately compromised, insulin-stimulating diets may improve follicle uniformity and thereby litter uniformity. If follicle development is hardly compromised at weaning, insulin-stimulating diets after weaning are not likely to further improve follicle development and thereby litter uniformity. Sow body condition loss during lactation suppresses follicle development at weaning, and young sows are more sensitive to adverse effects of body condition loss on follicle development than older sows.

Both IGF-1 levels after weaning and follicle development at weaning, thus, are related to sow parity and sow body condition loss during lactation. Both potential hypotheses are schematically summarized in Table 8.5. These hypotheses, however, clearly warrant further validation in controlled experiments.

Table 8.5 Potential hypotheses regarding effects of insulin-stimulating diets during the weaning-to-estrus interval related to sow body condition loss during preceding lactation, follicle development at weaning and IGF-1 status at weaning for young and older sows

	Sow body condition loss during preceding lactation		
	Low	Moderate	High
Young sows			
Follicle development at weaning[1]	-	--	---
IGF-1 status at weaning[2]	+++	++	+
Expected effect of insulin-stimulating diet during WEI[3]			
On ovulation rate or embryo survival	~	+/~	+
On litter uniformity	+	~/+	~
Older sows			
Follicle development at weaning	+	-	--
IGF-1 status at weaning	+	+/-	-
Expected effect of insulin-stimulating diet during WEI			
On ovulation rate or embryo survival	~	~	~
On litter uniformity	~	+ or ~?	~ or +?

[1] ---, highly compromised; --, moderately compromised; -, low compromised; +, not compromised.
[2] +++, very high; ++, high; +, moderate; -, low.
[3] +, positive effect; ~, no effect.
WEI, weaning-to-estrus interval.

8.3.4 Possible effects of insulin-stimulating diets during lactation

In sows with compromised follicle development at the end of lactation, insulin-stimulating diets during lactation are expected to be beneficial for subsequent litter uniformity, because (i) insulin-stimulating diets during lactation will enhance insulin and IGF-1 levels during lactation, and thereby also IGF-1 levels after weaning; and (ii) both insulin and IGF-1 levels during lactation are positively related to follicle development around weaning (see Chapter 1). The importance of sow metabolic state during a conventional 3 - 4 week lactation, and thereby plasma insulin and IGF-1 levels, for subsequent litter uniformity is also confirmed in this thesis (see Paragraph 8.2). Effects on piglet birth weight and litter uniformity were most pronounced in the study of Van den Brand et al. (2009), in which an insulin-stimulating diet was fed during a 27-days lactation plus the weaning-to-estrus interval (Table 8.4). Recently, Chen et al. (*in press*) showed that substituting 1 kg of the lactation diet with a sugar-rich supplement during the last 9 days of a 28-days lactation in first parity sows resulted in larger subsequent litters (+ 1.5 piglet; $P = 0.04$) with comparable litter uniformity when uncorrected for these litter size differences (SD of birth weights

were 268 ± 21 and 262 ± 23 g for the sugar-rich supplement and control sows, respectively; birth weight CV was not reported). This may indicate a relatively higher litter uniformity in the sows that received the sugar-rich supplement during late lactation. It needs further study, however, whether enhanced plasma insulin and IGF-1 levels or insulin-stimulating diets during lactation are additive to or interact with enhanced insulin and IGF-1 levels or insulin-stimulating diets during the weaning-to-estrus interval. Based on studies of Zak et al. (1997a, b), who demonstrated that follicle development and oocyte quality are most sensitive to metabolic conditions (using feed restriction) and thereby plasma insulin and IGF-1 levels during the last week of a 3 - 4 week lactation, it is speculated that especially the last week of a conventional 3 - 4 week lactation is important for follicle and oocyte development, and thereby subsequent litter uniformity.

8.3.5 Summarizing

Plasma insulin levels during the weaning-to-estrus interval, but not plasma IGF-1 levels, can be effectively enhanced by dietary sugars and starch in a dose-dependent manner. The physiological experiments in this thesis do not confirm that nutritionally increased plasma insulin and IGF-1 levels during only the weaning-to-estrus interval improve follicle development and uniformity and thereby subsequent litter uniformity in multiparous sows. This may be related to the (early) prenatal evaluation of litter uniformity and/or the short weaning-to-estrus interval and ongoing genetic improvements in modern sows. Furthermore, it is hypothesized that plasma IGF-1 levels after weaning may have been insufficient and/or follicle development at weaning may have been insufficiently compromised for the insulin-stimulating diets to exert positive effects on follicle development and subsequent litter uniformity. Both plasma IGF-1 levels after weaning and follicle development at weaning are related to sow parity and sow body condition loss during lactation, and these sow factors thus may influence effects of insulin-stimulating diets during the weaning-to-estrus interval. These hypotheses, as well as effects of insulin-stimulating diets during lactation, warrant further study in controlled experiments.

8.4 GENERAL CONCLUSIONS AND RECOMMENDATIONS

Piglet birth weight and litter uniformity are important factors for pre-weaning piglet survival, especially in large litters with high competition among littermates. Marginal improvements in piglet birth weight or litter uniformity can have substantial effects on piglet survival; for every 100 g increase in mean piglet birth weight, live-born piglet survival during the first 3 days after birth increased with 3.1%, whereas for every 1% reduction in within-litter birth weight CV, early piglet survival increased with 1.1%. Both piglet birth weight (- 40 to 41 g/piglet) and litter uniformity (+ 0.8% in birth weight CV/piglet) decreased with an increase in litter size. With increasing litter sizes in the future, therefore, more attention is needed for factors that control piglet birth weight and litter uniformity.

For the first time it is shown that pre-mating conditions related to sow metabolic state can affect subsequent litter uniformity. Litter uniformity was improved in sows with a prolonged weaning-to-pregnancy interval: litter uniformity was significantly higher in sows with a weaning-to-pregnancy interval of > 21 days (- 1.7% in birth weight CV; including repeat breeders), and numerically higher in sows with a weaning-to-pregnancy interval between 8 and 21 days (- 1.4% in birth weight CV) than in sows with a weaning-to-pregnancy interval of ≤ 7 days. In sows with a regular weaning-to-pregnancy interval (≤ 7 days), litter uniformity at birth was negatively and linearly related to body condition loss during previous lactation: an increase in body weight loss during previous lactation from ≤ 3.5% (20% lowest class) to > 13% (20% highest class) resulted in a 28 g-increase in birth weight SD, and an increase in backfat loss during previous lactation from ≤ 2 mm (20% lowest class) to > 5 mm (20% highest class) resulted in a 25 g-increase in birth weight SD and a 1.8%-increase in birth weight CV. These results indicate that body condition loss of sows during lactation should be minimized or otherwise the weaning-to-pregnancy interval could be prolonged in order to assure good litter uniformity in the subsequent litter. Moreover, these results confirm that litter uniformity at birth is already partly determined during the pre-mating period, likely related to (insufficient) restoration of follicle development, and possibly mediated by insulin and/or IGF-1.

Plasma insulin levels during the weaning-to-estrus interval can be effectively enhanced by dietary sugars as dextrose and sucrose (fast and high peaks directly after feeding) and starch (long-term enhanced insulin levels at ~ 4 hours after feeding) in a dose-dependent manner, but effects of fermentable carbohydrate sources as sugarbeet pulp and lactose on (long-term) insulin secretion seem very limited. Increasing the feeding frequency without

increasing total daily feed intake is not a proper way to stimulate insulin secretion, because insulin responses remain relatively lower after feeding smaller meal sizes.

Plasma IGF-1 levels during the first days after weaning can not be effectively stimulated by insulin-stimulating diets during the weaning-to-estrus interval, which indicates that factors other than insulin are limiting for IGF-1 production in these first days after weaning. Insulin-stimulating diets during the weaning-to-estrus interval possibly result in higher plasma IGF-1 levels from day 4 - 5 after weaning onwards. Due to this possible latency in IGF-1 response to insulin-stimulating diets after weaning, however, the weaning-to-estrus interval seems too short for an effective stimulation of IGF-1 by insulin-stimulating diets.

Results of this thesis do not confirm that insulin-stimulating diets during the weaning-to-estrus interval can improve follicle development and subsequent litter uniformity of embryos, fetuses and placentas, or piglets, in multiparous sows. It furthermore remains unclear whether insulin-stimulating diets exert their effects on follicle development through effects on plasma insulin levels or on plasma IGF-1 levels. It is hypothesized that insulin-stimulating diets during the weaning-to-estrus interval may only be beneficial for follicle development and thereby subsequent litter uniformity in combination with sufficiently high plasma IGF-1 levels after weaning and/or a low to moderately compromised follicle development at weaning. If follicle development at weaning is low to moderately compromised, insulin-stimulating diets after weaning may improve litter uniformity, whereas in sows with highly compromised follicle development at weaning, insulin-stimulating diets after weaning are expected to restore reduced ovulation rates and embryo survival rather than improve litter uniformity.

It is therefore recommended to further study the role of plasma IGF-1 levels and follicle development at weaning, which are both related to sow parity and sow body condition loss during lactation. Attention should be given to increasing IGF-1 levels during (late) lactation, either through reducing sow body condition loss during lactation or through insulin-stimulating diets during lactation. Moreover, it is recommended to further study whether an insulin-stimulating diet during (late) lactation could improve subsequent litter uniformity, and whether its effects are additive to or interact with effects of an insulin-stimulating diet after weaning.

Feeding strategies, i.e. feeding frequency and diet composition, which modulate the pattern of insulin secretion during the day (frequent short peaks/day vs. less frequent sustained

peaks/day) rather than total daily insulin secretion may influence follicle development and subsequent development and uniformity of embryos and piglets. This thesis, however, did not give conclusive insight in how different insulin secretion patterns during the day are related to follicle development. It needs further study, therefore, which insulin secretion patterns are optimal for good follicle development, and how these insulin patterns can be achieved.

In order to develop fine-tuned pre-mating feeding strategies that can assist farmers in improving litter uniformity, an improved understanding of the conditions, such as sow parity and sow body condition loss as well as feeding frequency, under which insulin-stimulating diets during lactation and/or during the weaning-to-estrus interval have beneficial effects on litter uniformity, is needed.

Finally, although effects of pre-mating nutritional and metabolic conditions on subsequent piglet birth weight and litter uniformity seem only marginal, these marginal effects can have substantial effects on pre-weaning piglet survival. This indicates the practical relevance of optimizing pre-mating nutritional and metabolic conditions, and thereby optimal follicle development, for piglet survival.

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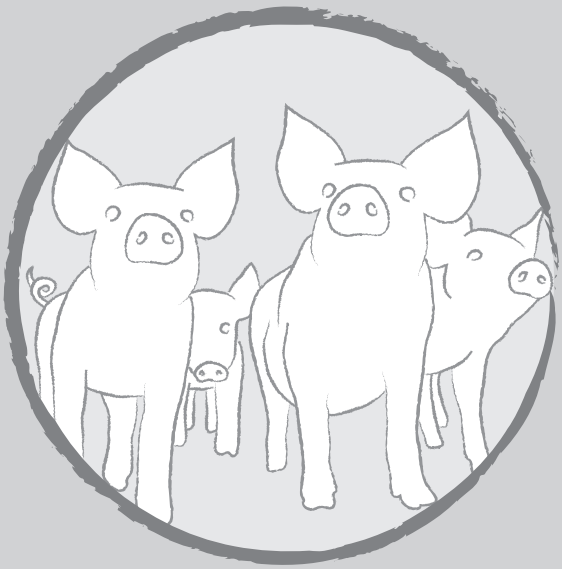
Summary



Over the last years, litter size has substantially increased to 13 - 15 total born piglets on average. This increase in litter size, however, is associated with an increase in pre-weaning piglet mortality. In nowadays European pig production, one out of five piglets born dies before weaning, which is a major economic and welfare problem. For piglet survival, as well as for piglet performance before and after weaning, high piglet birth weights and litter uniformity are crucial. Although consequences of piglet birth weight and litter uniformity on piglet survival and performance have been studied, the (biological) causes of within-litter variation and possible factors affecting piglet birth weight and litter uniformity are poorly described. Both piglet birth weight and litter uniformity are affected by litter size, parity and breed, at least in sow populations with average litter sizes up to 13 - 14 total born piglets, but there may be other factors involved.

Within-litter variation in birth weight seems to be the consequence of within-litter variation in early embryo development, which in turn reflects variation in follicle and oocyte development. Recent studies suggest that pre-mating sow diets can influence development and uniformity of fetuses and piglets, probably related to the insulin and Insulin-like Growth Factor-1 (IGF-1) stimulating effects of these diets. Insulin and IGF-1 are known to stimulate follicle and oocyte development, either indirectly via stimulation of gonadotropin release or directly at the ovarian level, where insulin and IGF-1 can act alone or by amplifying gonadotropin action. Besides effects of pre-mating diets, plasma insulin and IGF-1 levels and also follicle development and subsequent embryo development are influenced by the pre-mating metabolic state of the sow.

The main hypothesis of this thesis is that piglet birth weight and litter uniformity are already (partly) determined in the pre-mating period. More specifically, it is hypothesized that pre-mating plasma insulin and IGF-1 levels affect follicle and oocyte development and uniformity, and thereby subsequent embryo development and uniformity and luteal development, subsequent placental and fetal development and uniformity and finally piglet birth weight and litter uniformity. Aims of this thesis are (i) to study effects of insulin-stimulating diets during the weaning-to-estrus interval on plasma insulin and IGF-1 levels, follicle development and uniformity, and consequences for embryo, fetal and placental development and uniformity and luteal development at different stages of pregnancy in sows; and (ii) to study effects of pre-mating conditions related to sow metabolic state during lactation and after weaning on subsequent piglet birth weight and litter uniformity.

Insulin-stimulating diets during the weaning-to-estrus interval

A series of physiological experiments was performed to study whether nutritionally increased plasma insulin and IGF-1 levels during the weaning-to-estrus interval could improve follicle development and uniformity, and subsequent embryo, fetal and placental development and uniformity in multiparous sows. Multiparous sows were used, because these sows have less uniform litters than younger sows.

In order to find the most suitable insulin-stimulating diet to create large contrasts in plasma insulin and IGF-1 levels among sows, effects of different (combinations of) dietary carbohydrate sources (fed twice daily) on plasma insulin and IGF-1 levels in anabolic non-pregnant multiparous sows were evaluated in **Chapter 2**. It was concluded that sugar sources as dextrose and sucrose have the potential to stimulate fast and high insulin peaks directly after feeding, especially when combined with additional lactose. The fermentable carbohydrate sources sugarbeet pulp and lactose did not result in the expected prolonged enhanced insulin levels after feeding. Modulation of plasma IGF-1 levels by dietary carbohydrates seems limited in anabolic, non-pregnant sows.

Based on the results of Chapter 2, the dextrose plus lactose (both 150 g/day) diet was subsequently used to study effects of nutritionally increased plasma insulin and IGF-1 levels during the weaning-to-estrus interval on plasma luteinizing hormone levels and follicle development [**Chapter 3**] and consequences for luteal development, progesterone levels and embryo development and uniformity at day 10 of pregnancy [**Chapter 4**]. Day 10 of pregnancy was chosen as the first evaluation moment to get a complete view on within-litter variation in embryo development prior to implantation; after day 10 of pregnancy, part of the within-litter variation is lost as a consequence of prenatal losses during the implantation period (~ day 12 - 16 of pregnancy). Unexpectedly, total insulin secretion and absolute insulin levels were not successfully stimulated, although contrasts were created in the insulin secretion patterns during the weaning-to-estrus interval by feeding the insulin-stimulating diet (dextrose plus lactose, both 150 g/day) at 4h-intervals (6 short insulin peaks/day) compared to an isocaloric control diet at 12h-intervals (2 sustained insulin peaks/day) at a similar daily feed allowance. Plasma IGF-1 levels during the 4-day weaning-to-estrus interval were not affected by the dietary treatments. The dextrose plus lactose diet fed at 4h-intervals during the weaning-to-estrus interval resulted in a lower pre-ovulatory LH surge, smaller and less uniform follicles at day 4 after weaning and subsequently smaller corpora lutea and numerically less developed embryos at day 10 of pregnancy compared

to the control diet fed at 12h-intervals. Independent of treatment, plasma insulin levels during the first three days after weaning were positively related to LH secretion, follicle diameter and subsequent progesterone levels during the first 10 days of pregnancy, and plasma insulin levels tended to be positively related to embryo development (diameter and DNA content), but not embryo uniformity, at day 10 of pregnancy. Whether and how the improved progesterone production and embryo development during early pregnancy result in a more uniform development of fetuses and piglets at later stages of pregnancy was the focus of Chapter 5.

In **Chapter 5**, consequences of different levels of insulin-stimulating feed components during the weaning-to-estrus interval for placental and fetal development and uniformity after the implantation period (at day 42 of pregnancy) were evaluated. Postprandial insulin responses, both fast insulin peaks and long-term insulin level (~ 4h after feeding), were successfully stimulated during the 4-day weaning-to-estrus interval by feeding insulin-stimulating diets supplemented with different levels of dextrose plus starch (both at 375 g/day or both at 172 g/day) compared to an isocaloric control diet twice a day, but plasma IGF-1 levels during the first three days after weaning were not affected. These insulin-stimulating diets, however, did not improve follicle development or subsequent development and uniformity of fetuses or placentas at day 42 of pregnancy. Plasma insulin and IGF-1 levels during the first three days after weaning were neither related to follicle development or subsequent fetal and placental development and uniformity, although a positive relation between the postprandial insulin response (as indicated by maximal insulin level after feeding) at day 2.5 after weaning and progesterone level at day 42 of pregnancy was found.

Because uniformity of embryos, fetuses or placentas was not influenced by the insulin-stimulating diets, nor related to plasma insulin and IGF-1 levels during the weaning-to-estrus interval, the physiological experiments in this thesis do not confirm that nutritionally increased plasma insulin and IGF-1 levels during only the weaning-to-estrus interval can improve follicle development and uniformity, and thereby subsequent litter uniformity in multiparous sows.

Pre-mating conditions related to sow metabolic state

To study whether pre-mating conditions related to sow metabolic state during lactation and after weaning could affect subsequent piglet birth weight and litter uniformity, different

sow models, known to affect plasma insulin and IGF-1 levels and follicle development, were used. In sows with severe body condition loss during lactation, plasma insulin and IGF-1 levels and follicle development at weaning are suppressed, and restoration of plasma insulin and IGF-1 levels and follicle development occurs in sows with a prolonged lactation or a recovery period after weaning (i.e. a prolonged weaning-to-pregnancy interval).

In **Chapter 6**, piglet birth weights and litter uniformity, and effects of pre-mating insulin-stimulating diets, were therefore studied in organic sows with prolonged lactations (6 weeks). These sows farrowed large litters of 17.4 ± 0.3 total born piglets, which likely reflects their improved follicle development at weaning compared to conventionally weaned sows (at 3 - 4 weeks of lactation). The insulin-stimulating diets (sucrose plus lactose; both 150 g/day) during only the weaning-to-estrus interval, or during the last two weeks of the 6-week lactation period plus the weaning-to-estrus interval, however, did not result in improved piglet birth weights or uniformity of the subsequent litter in these organic sows, possibly also related to their restored follicle development at weaning. In these large organic litters, piglet birth weight and litter uniformity were strongly related to piglet survival during lactation, as they do in conventional smaller litters. Regression coefficients indicate that for every 100 g increase in mean piglet birth weight, live-born piglet survival during the first 3 days after birth increased with 3.1%, whereas for every 1% reduction in within-litter birth weight coefficient of variation (CV), early piglet survival increased with 1.1%. This indicates that even marginal improvements in piglet birth weight or litter uniformity can have substantial effects on piglet survival.

In **Chapter 7**, effects of a prolonged weaning-to-pregnancy interval (used as a model for a recovery period after weaning) and effects of body condition loss during lactation in sows with a regular weaning-to-pregnancy interval (≤ 7 days) on subsequent piglet birth weight and litter uniformity were studied in sows with conventional 3 - 4 week lactations. Litter uniformity at birth was significantly higher in sows with a weaning-to-pregnancy interval of > 21 days (- 1.7% in birth weight CV; including repeat breeders), and numerically higher in sows with a weaning-to-pregnancy interval between 8 and 21 days (- 1.4% in birth weight CV) than in sows with a weaning-to-pregnancy interval of ≤ 7 days. In sows with a regular weaning-to-pregnancy interval (≤ 7 days), litter uniformity at birth was negatively and linearly related to body condition loss during previous lactation; for example, an increase in body weight loss during previous lactation from ≤ 3.5% (20% lowest class) to > 13% (20% highest class) resulted in + 28 g in birth weight SD, and an increase in backfat loss during previous lactation from ≤ 2 mm (20% lowest class) to > 5 mm (20% highest class)

resulted in + 25 g in birth weight SD and + 1.8% in birth weight CV. These results show that litter uniformity is compromised by severe sow body condition loss during lactation and improved in sows with a prolonged weaning-to-pregnancy interval, likely related to (insufficient) restoration of follicle development.

General conclusions and recommendations

Piglet birth weight and litter uniformity are important factors for pre-weaning piglet survival, especially in large litters with high competition among littermates. Therefore, more attention is needed for factors that control piglet birth weight and litter uniformity.

For the first time it is shown that pre-mating conditions related to sow metabolic state can affect subsequent litter uniformity. Litter uniformity at birth was compromised by severe sow body condition loss during previous lactation and improved in sows with a prolonged weaning-to-pregnancy interval. These results indicate that body condition loss of sows during lactation should be minimized or otherwise the weaning-to-pregnancy interval could be prolonged in order to assure good litter uniformity in the subsequent litter. Moreover, these results confirm that litter uniformity at birth is already (partly) determined during the pre-mating period, likely related to (insufficient) restoration of follicle development.

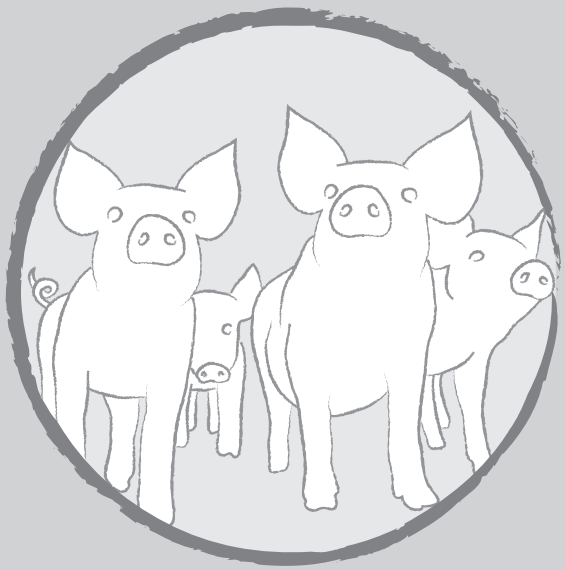
Plasma insulin levels during the weaning-to-estrus interval can be effectively enhanced by dietary sugars as dextrose and sucrose (fast and high peaks directly after feeding) and starch (long-term enhanced insulin levels at ~ 4h after feeding) in a dose-dependent manner, but effects of fermentable carbohydrate sources on (long-term) insulin secretion seem very limited. Increasing the feeding frequency without increasing total daily feed intake is not a proper way to stimulate insulin secretion. Plasma IGF-1 levels during the first days of the weaning-to-estrus interval cannot be effectively stimulated by insulin-stimulating diets in this period.

Results of this thesis do not confirm that insulin-stimulating diets during the weaning-to-estrus interval can improve follicle development and subsequent litter uniformity of embryos, fetuses and placentas, or piglets, in multiparous sows. It is hypothesized that insulin-stimulating diets during the weaning-to-estrus interval may only be beneficial for follicle development and thereby subsequent litter uniformity in combination with sufficiently high plasma IGF-1 levels after weaning and/or a low to moderately compromised follicle development at weaning. It is therefore recommended to further

study the role of plasma IGF-1 levels and follicle development at weaning, which are both related to sow parity and sow body condition loss during lactation. Attention should be given to increasing IGF-1 levels during (late) lactation, either through reducing sow body condition loss during lactation or through insulin-stimulating diets during lactation. Moreover, it is recommended to further study whether an insulin-stimulating diet during (late) lactation could improve subsequent litter uniformity, and whether its effects are additive to or interact with effects of an insulin-stimulating diet after weaning.

Finally, although effects of pre-mating nutritional and metabolic conditions on subsequent piglet birth weight and litter uniformity seem only marginal, these marginal effects can have substantial effects on pre-weaning piglet survival. This indicates the practical relevance of optimizing pre-mating nutritional and metabolic conditions, and thereby optimal follicle development, for piglet survival.

Samenvatting



Door genetische selectie en verbetering van het management is de toomgrootte van zeugen (het aantal biggen dat geboren wordt per worp) de laatste jaren flink gestegen en momenteel worden tomen van gemiddeld 13 tot 15 biggen geboren. Tegelijkertijd zijn echter het gemiddelde geboortegewicht van biggen en de uniformiteit van geboortegewichten binnen een toom (ofwel toomuniformiteit) gedaald, en is de sterfte van biggen tijdens de zoogperiode (van 3 à 4 weken) gestegen. In de huidige Europese varkenshouderij sterft momenteel gemiddeld één op de vijf biggen al vóór het spenen, wat een groot welzijns- en economisch probleem is. Voor een goede bigoverleving, maar ook voor een goede groei tot slacht en een goede vleeskwaliteit, zijn hoge geboortegewichten en toomuniformiteit belangrijk. Hoewel de consequenties van geboortegewicht en toomuniformiteit voor overleving en groei van biggen al zijn onderzocht, zijn de (biologische) oorzaken van variatie in geboortegewicht en toomuniformiteit slecht beschreven.

Foetale groei, en daarmee het geboortegewicht van een big, wordt grotendeels bepaald door de opname van voedingsstoffen via de placenta, welke op zijn beurt grotendeels samenhangt met de oppervlakte waarmee het embryo zich aanhecht aan de baarmoederwand (ofwel placenta-oppervlak). Hoe groter het placenta-oppervlak, des te beter de ontwikkeling van de embryo's en foetussen. Het placenta-oppervlak ligt al grotendeels vast op ~ dag 35 van de dracht (de totale draagtijd van het varken is ~115 dagen). De binnen-toom variatie in placenta-oppervlak in het varken hangt samen met de binnen-toom variatie in embryonale ontwikkeling tijdens de aanhechtingsfase op ~ dag 12 van de dracht. Beter ontwikkelde embryo's zullen namelijk meer plaats innemen in de baarmoeder, wat resulteert in een groter placenta-oppervlak. Minder ontwikkelde embryo's sterven af tijdens de aanhechtingsfase (omdat ze niet op tijd een plek kunnen innemen in de baarmoeder) of hebben een relatief klein aanhechtingsoppervlak, wat resulteert in een klein placenta-oppervlak. Binnen-toom variatie in embryonale ontwikkeling is met name het gevolg van variatie in follikel- en eicelontwikkeling. Samenvattend lijkt binnen-toom variatie in geboortegewichten dus het gevolg te zijn van variatie in follikel- en eicelontwikkeling, wat resulteert in binnen-toom variatie in embryonale ontwikkeling en daarmee in placenta-oppervlak.

Recent onderzoek laat zien dat de voeding van de zeug in de periode vóór het dekken (voorgaande zoogperiode en/of het interval spenen-dekken)[1], de periode waarin de

1 Het spenen, oftewel het weghalen van de biggen en daarmee het beëindigen van de zoogperiode, is het signaal voor het begin van een nieuwe reproductiecyclus. De voortplantingshormonen LH en FSH worden in hogere mate afgegeven en follikels gaan uitgroeien. Normaal gesproken worden zeugen binnen 4 tot 7 dagen na het spenen berig, worden dan geïnsemineerd en vindt ook de eisprong plaats. De ontwikkeling van de follikels die tot eisprong komen na het spenen begint al tijdens de voorgaande zoogperiode.

follikels en eicellen zich ontwikkelen, van invloed kan zijn op de ontwikkeling en uniformiteit van foetussen en biggen in de volgende worp. Waarschijnlijk hangt dit samen met het stimulerende effect van de gebruikte voeders op insuline (hormoon dat een rol speelt in de bloedsuikerregulatie) en Insulin-like Growth Factor-1 (IGF-1; een groeihormoon) gehaltes in het bloed. Insuline en IGF-1 beïnvloeden de afgifte van de voortplantingshormonen (LH en FSH), die vervolgens de groei en ontwikkeling van follikels stimuleren. Bovendien kunnen insuline en IGF-1 ook rechtstreeks de groei en ontwikkeling van follikels stimuleren. Gehaltes van insuline en IGF-1 in het bloed en follikelontwikkeling worden niet alleen beïnvloed door het voer, maar ook door de energiebalans[2] van de zeug.

De belangrijkste hypothese van dit proefschrift is dat geboortegewichten en toomuniformiteit van biggen al (deels) worden bepaald in de periode vóór het dekken. Meer specifiek is de hypothese dat insuline- en IGF-1-gehaltes in het bloed in de periode vóór het dekken de ontwikkeling van follikels kunnen stimuleren. Wanneer follikels meer gestimuleerd worden, ontstaan naar verwachting beter ontwikkelde en meer uniforme eicellen, die na de bevruchting uitgroeien tot beter ontwikkelde en meer uniforme embryo's en foetussen. Dit kan uiteindelijk resulteren in meer uniforme tomen. Ook ontwikkelen de follikels zich na de eisprong tot zogenaamde 'gele lichamen', die progesteron produceren. Progesteron is essentieel voor de ontwikkeling van embryo's. Een betere follikelontwikkeling zal waarschijnlijk ook leiden tot een betere ontwikkeling van de gele lichamen die daardoor meer progesteron gaan produceren, wat de ontwikkeling van embryo's en foetussen verder bevordert. Het doel van dit onderzoek is het bestuderen van (i) effecten van insuline-stimulerende voeders tijdens het interval spenen-dekken op insuline- en IGF-1-gehaltes in het bloed en op follikelontwikkeling, en de consequenties voor embryonale, foetale en placentaontwikkeling en -uniformiteit en ontwikkeling van de gele lichamen op verschillende stadia van de dracht in zeugen; en (ii) effecten van omstandigheden vóór het dekken die samenhangen met de energiebalans van de zeug op geboortegewichten en toomuniformiteit van biggen in de daaropvolgende worp.

2 De balans tussen energieopname en energieverbruik. Als de energieopname lager is dan het energieverbruik, resulteert dat in een negatieve energiebalans en worden de energievoorraden in het lichaam aangesproken, wat resulteert in conditieverlies. Dit geldt bijvoorbeeld voor zeugen tijdens de zoogperiode. Als de energieopname hoger is dan het energieverbruik, resulteert dat in een positieve energiebalans, wat resulteert in groei. Dit geldt bijvoorbeeld voor zeugen na de zoogperiode.

Insuline-stimulerende voeders tijdens het interval spenen-dekken

In meerdereworps zeugen is een serie experimenten uitgevoerd om te onderzoeken of via voeding verhoogde insuline- en IGF-1-gehaltes in het bloed tijdens het interval spenen-dekken invloed hebben op follikelontwikkeling en vervolgens op ontwikkeling en uniformiteit van embryo's, foetussen en placenta's.

Voordat de effecten van voeding op bovenstaande reproductiekenmerken zijn onderzocht, is eerst bepaald welke voercomponenten het meest geschikt zijn om contrasten te creëren in insuline- en IGF-1-gehaltes in het bloed. Daartoe zijn de effecten van verschillende (combinaties van) koolhydraatbronnen op insuline- en IGF-1-gehaltes in het bloed onderzocht in niet-drachtige zeugen met een positieve energiebalans [**Hoofdstuk 2**]. Concluderend hebben suikerbronnen zoals dextrose en sucrose de hoogste potentie om insuline-afgifte te stimuleren en hoge insulinepieken direct na het voeren te veroorzaken, vooral in combinatie met lactose. De fermenteerbare koolhydraatbronnen, suikerbietenpulp en lactose, resulteerden niet in de verwachte langdurig verhoogde insulinegehaltes na het voeren. Het effect van de verschillende koolhydraatbronnen in het voer op IGF-1-gehaltes in het bloed lijkt klein in zeugen met een positieve energiebalans.

Gebaseerd op de resultaten van Hoofdstuk 2 is een volgend experiment uitgevoerd met het dextrose-plus-lactosevoer (beide 150 g/dag). In dit experiment is gekeken naar de effecten van via het voer verhoogde insuline- en IGF-1-gehaltes in het bloed tijdens het interval spenen-dekken op LH-gehaltes in het bloed en follikelontwikkeling [**Hoofdstuk 3**] en vervolgens op de ontwikkeling van de gele lichamen en progesterongehaltes in het bloed en op de embryonale ontwikkeling en uniformiteit op dag 10 van de dracht [**Hoofdstuk 4**]. Dag 10 van de dracht is gekozen als eerste evaluatiemoment om een compleet overzicht te krijgen van de binnen-toom variatie in embryonale ontwikkeling; na dag 10 gaat namelijk een deel van de binnen-toom variatie verloren door het afsterven van embryo's tijdens de aanhechtingsfase (~ dag 12-16 van de dracht). Tijdens het interval spenen-dekken werden positieve relaties gevonden tussen de insulinegehaltes in het bloed enerzijds en LH-afgifte in het bloed en follikelontwikkeling anderzijds. De insulinegehaltes in het bloed tijdens het interval spenen-dekken waren ook positief gerelateerd aan progesterongehaltes in het bloed en embryonale ontwikkeling tijdens de eerste 10 dagen van de dracht, maar er werden geen relaties gevonden met de uniformiteit van de embryo's. Óf en hoe de verbeterde progesterongehaltes en embryonale ontwikkeling tijdens de vroege dracht vervolgens kunnen leiden tot een betere en meer uniforme

ontwikkeling van foetussen en biggen in latere stadia van de dracht was daarom de focus van Hoofdstuk 5.

In **Hoofdstuk 5** zijn de effecten onderzocht van verschillende niveaus van insuline-stimulerende voercomponenten tijdens het interval spenen-dekken op de ontwikkeling en uniformiteit van foetussen en placenta's (op dag 42 van de dracht). Drie voeders zijn vergeleken, waarvan twee voeders gesupplementeerd waren met dextrose plus zetmeel (beide 375 g/dag of beide 172 g/dag; insuline-stimulerende voeders) en een controlevoer. De insuline-afgifte na het voeren, zowel de insuline-piekhoogte (binnen 1 uur na voeren) als langdurige insulinegehaltes (~ 4 uur na het voeren), tijdens het 4-daagse interval spenen-dekken waren verhoogd door de insuline-stimulerende voeders ten opzichte van het controlevoer. De IGF-1-gehaltes in het bloed tijdens de eerste 3 dagen na spenen waren niet beïnvloed door de 3 voerbehandelingen. De insuline- en IGF-1-gehaltes in het bloed tijdens de eerste 3 dagen van het interval spenen-dekken waren niet gerelateerd aan follikelontwikkeling of ontwikkeling en uniformiteit van de foetussen en placenta's op dag 42 van de dracht. Wel was er een positieve relatie tussen insulinegehaltes in het bloed tijdens het interval spenen-dekken en progesterongehaltes in het bloed op dag 42 van de dracht.

De uniformiteit van embryo's, foetussen en/of placenta's lijkt in deze experimenten dus niet samen te hangen met de insuline- en IGF-1-gehaltes in het bloed tijdens het interval spenen-dekken. Hiermee kan de hypothese dat via het voer verhoogde insuline- en IGF-1-gehaltes in het bloed tijdens het interval spenen-dekken kunnen leiden tot een verbeterde follikelontwikkeling en vervolgens een verbeterde toomuniformiteit in meerdereworps zeugen niet worden bevestigd in dit proefschrift.

Omstandigheden vóór het dekken die samenhangen met de energiebalans van de zeug

In zeugen die veel conditie (lichaamsgewicht en spek) verliezen tijdens de zoogperiode zijn zowel insuline- en IGF-1-gehaltes in het bloed als follikelontwikkeling onderdrukt. Herstel van zowel insuline- en IGF-1-gehaltes in het bloed als follikelontwikkeling treedt op in zeugen met een verlengde zoogperiode of een herstelperiode na het spenen (ofwel een verlengd interval spenen-dracht). Deze drie zeugmodellen (conditieverlies tijdens de zoogperiode, verlengde zoogperiode, verlengd interval spenen-dracht) zijn gebruikt om te onderzoeken of omstandigheden vóór het dekken die samenhangen met de energiebalans

van de zeug de geboortegewichten en toomuniformiteit van biggen in de daaropvolgende worp kunnen beïnvloeden.

In **Hoofdstuk 6** zijn effecten van insuline-stimulerende voeders vóór het dekken op geboortegewichten en toomuniformiteit van biggen bestudeerd in biologische zeugen met verlengde zoogperiodes (6 weken). Deze zeugen wierpen grote tomen van 17.4 ± 0.3 totaal geboren biggen. De insuline-stimulerende voeders (sucrose plus lactose, beide 150 g/dag) tijdens het interval spenen-dekken, of tijdens de laatste 2 weken van de zoogperiode plus het interval spenen-dekken, leidden niet tot een verbetering van geboortegewichten en toomuniformiteit van biggen in de daaropvolgende worp. Dit hangt mogelijk samen met een herstelde follikelontwikkeling bij het spenen na een 6-weekse zoogperiode waarin de zeugen uiteindelijk vrijwel geen gewicht verloren. In deze grote tomen waren het geboortegewicht en de toomuniformiteit van biggen sterk gerelateerd aan de bigoverleving tijdens de zoogperiode. Voor elke 100 g toename in het gemiddelde geboortegewicht, nam de bigoverleving tijdens de eerste 3 dagen na geboorte toe met 3,1%. Voor elke 1% afname in binnen-toom variatie in geboortegewicht, zoals aangeduid met de binnen-toom variatiecoëfficiënt (CV), nam de bigoverleving tijdens de eerste 3 dagen na geboorte toe met 1,1%. Dit geeft aan dat zelfs kleine verbeteringen in geboortegewichten en toomuniformiteit van biggen substantiële effecten kunnen hebben op bigoverleving.

In **Hoofdstuk 7** zijn in zeugen met gangbare 3-4 weekse zoogperiodes de effecten onderzocht van een verlengd interval spenen-dracht (gebruikt als model voor een herstelperiode na de zoogperiode) en van conditieverlies tijdens de zoogperiode in zeugen met een regulier interval spenen-dracht (≤ 7 dagen) op geboortegewichten en toomuniformiteit van biggen in de daaropvolgende worp. Toomuniformiteit van biggen bij geboorte was hoger in zeugen met een interval spenen-dracht van meer dan 21 dagen (-1,7% in geboortegewicht CV) en tussen de 8 en 21 dagen (-1,4% in geboortegewicht CV) vergeleken met zeugen met een regulier interval spenen-dracht van minder dan 7 dagen. In zeugen met een regulier interval spenen-dracht hing de toomuniformiteit van biggen bij geboorte negatief en lineair samen met het conditieverlies van de zeug tijdens de voorgaande zoogperiode. Bijvoorbeeld, een toename in gewichtsverlies van de zeug tijdens de voorgaande zoogperiode van minder dan 3,5% (20% laagste klasse) tot meer dan 13% (20% hoogste klasse) resulteerde in een toename van 28 g in de binnen-toom standaarddeviatie (SD) in geboortegewicht. Een toename in spekverlies van de zeug tijdens de voorgaande zoogperiode van minder dan 2 mm (20% laagste klasse) tot meer dan 5 mm (20% hoogste klasse) resulteerde in een toename van 25 g in geboortegewicht SD en 1,8% in geboortegewicht CV. Deze resultaten

geven aan dat toomuniformiteit van biggen bij geboorte verslechterd is in zeugen met veel conditieverlies tijdens de voorgaande zoogperiode en verbeterd is in zeugen met een verlengd interval spenen-dracht (ofwel een herstelperiode na de zoogperiode). Deze effecten hangen mogelijk samen met (onvoldoende) herstel van follikelontwikkeling.

Algemene conclusies en aanbevelingen

Geboortegewichten en toomuniformiteit van biggen zijn belangrijke factoren voor bigoverleving tijdens de zoogperiode, vooral in grote tomen met veel competitie tussen toomgenoten. Daarom is meer aandacht nodig voor factoren die van invloed zijn op geboortegewichten en toomuniformiteit van biggen.

Voor het eerst is aangetoond dat omstandigheden vóór het dekken die samenhangen met de energiebalans van de zeug invloed kunnen hebben op toomuniformiteit van biggen in de daaropvolgende worp. Toomuniformiteit van biggen bij geboorte was verslechterd in zeugen met veel conditieverlies tijdens de voorgaande zoogperiode en verbeterd in zeugen met een verlengd interval spenen-dracht. Deze resultaten geven aan dat voor een goede toomuniformiteit van biggen het conditieverlies van de zeug tijdens de voorgaande zoogperiode geminimaliseerd moet worden of anders het interval spenen-dracht verlengd kan worden. Bovendien bevestigen deze resultaten dat toomuniformiteit van biggen bij geboorte al (deels) wordt bepaald in de periode vóór het dekken, wat mogelijk samenhangt met (onvoldoende) herstel van follikelontwikkeling.

Insulinegehaltes in het bloed tijdens het interval spenen-dekken kunnen effectief verhoogd worden met de voercomponenten dextrose en sucrose (hoge insulinepieken direct na voeren) en zetmeel (langdurig verhoogde insulinegehaltes op ~ 4 uur na voeren) en nemen toe met de toegevoegde hoeveelheid van deze voercomponenten. Effecten van fermenteerbare koolhydraatbronnen op (langdurige) insuline-afgifte lijken erg beperkt. De IGF-1-gehaltes in het bloed tijdens de eerste dagen van het interval spenen-dekken kunnen niet effectief gestimuleerd worden met insuline-stimulerende voeders tijdens deze periode.

De resultaten van dit proefschrift bevestigen niet dat insuline-stimulerende voeders tijdens het interval spenen-dekken de follikelontwikkeling, en daarmee toomuniformiteit van embryo's, foetussen, placenta's en/of biggen, kunnen verbeteren in meerdereworps zeugen. Een nieuwe hypothese is dat insuline-stimulerende voeders tijdens het interval spenen-dekken alleen een positief effect hebben op follikelontwikkeling en daarmee toomuniformiteit in combinatie met voldoende hoge IGF-1-gehaltes in het bloed en/of

een bepaalde mate van onderdrukte follikelontwikkeling bij spenen. Het wordt aanbevolen om de rol van IGF-1-gehaltes in het bloed en follikelontwikkeling bij spenen, welke beide samenhangen met het worpnummer en het conditieverlies van de zeug tijdens de zoogperiode, verder te onderzoeken. Bovendien wordt aanbevolen om verder te onderzoeken of insuline-stimulerende voeders tijdens (de laatste fase van) de zoogperiode de toomuniformiteit van de daaropvolgende worp kunnen verbeteren.

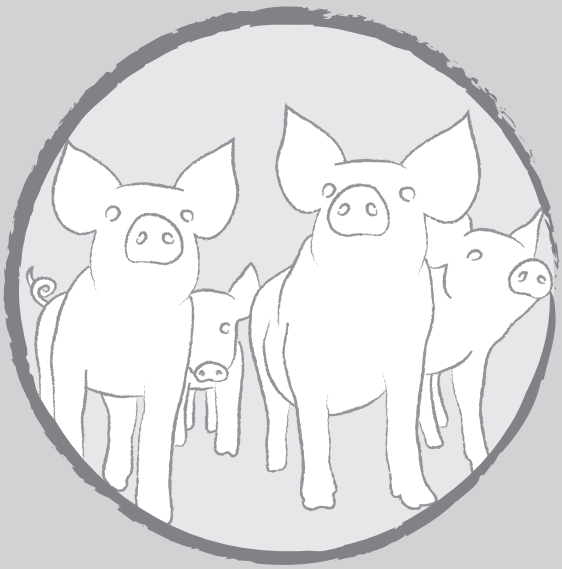
Tot slot, al lijken de effecten van voeding en energiebalans van de zeug vóór het dekken op geboortegewichten en toomuniformiteit van biggen in de daaropvolgende worp klein, deze kleine effecten kunnen een substantieel effect hebben op bigoverleving tijdens de zoogperiode. Dit benadrukt de praktische relevantie van een optimale voeding en energiebalans van de zeug vóór het dekken, en daarmee optimale follikelontwikkeling, voor bigoverleving.

About the author

Curriculum Vitae

Publications

Training and Supervision Plan



CURRICULUM VITAE

Johanna Gerardina Maria (Anne) Wientjes was born on the third of April 1985 in Boxmeer, and was raised in Sint Anthonis, The Netherlands. In 2003, after graduating from high school Elzendaalcollege in Boxmeer, she started her study Animal Sciences at Wageningen University and specialized in Quantitative Veterinary Epidemiology and Adaptation Physiology. For the specialization Quantitative Veterinary Epidemiology, she evaluated the effects of an intervention study at veterinarians to promote udder health at Dutch dairy farms, which was an assignment of the Dutch Udder Health Centre (UGCN) and the Dutch Animal Health Service (GD Deventer). For the specialization Adaptation Physiology, she studied the loin muscle as a measure of sow condition and its relation with reproductive performance of second parity sows. In addition, she completed an internship abroad at the 'Department of Primary Industries' (DPI) in Kyabram, Victoria, Australia, where she investigated effects of stage of lactation and feeding level on feed conversion efficiency in pasture-based Victorian dairying in the light of extended lactation. After her graduation in 2008, she started her PhD research at the Adaptation Physiology Group of Wageningen University aimed at improvement of piglet birth weight and litter uniformity, and thereby piglet survival. The results of her PhD research are presented in this thesis.

Johanna Gerardina Maria (Anne) Wientjes werd geboren op 3 april 1985 in Boxmeer en groeide op in Sint Anthonis. In 2003 behaalde zij haar VWO-diploma aan het Elzendaalcollege in Boxmeer. In datzelfde jaar begon zij aan de studie Dierwetenschappen aan Wageningen University, met als specialisaties Kwantitatieve Veterinaire Epidemiologie en Adaptatiefysiologie. Voor de specialisatie Kwantitatieve Veterinaire Epidemiologie evalueerde zij de effecten van een interventiestudie bij dierenartsenpraktijken ter verbetering van de uiergezondheid op melkveebedrijven, in opdracht van het Uiergezondheidscentrum Nederland (UGCN) en de Gezondheidsdienst voor Dieren (GD Deventer). Voor de specialisatie Adaptatiefysiologie deed zij onderzoek naar de dikte van de karbonadespier als maat voor de conditie van de zeug en de relatie met de reproductie resultaten van tweedeworps zeugen. Haar stage-opdracht heeft zij uitgevoerd bij 'The Department of Primary Industries' (DPI) in Kyabram, Victoria, Australië, waar zij onder andere onderzoek deed naar de effecten van lactatiestadium en voerniveau op de voer-efficiëntie van melkkoeien tijdens verlengde lactaties in seizoensgebonden melkveehouderij in Victoria. Na haar afstuderen in 2008 begon zij als promovenda bij de leerstoelgroep

Adaptatiefysiologie van Wageningen University aan een promotieonderzoek gericht op het verbeteren van geboortegewichten en uniformiteit, en daarmee overleving, van biggen. De resultaten van dit onderzoek zijn beschreven in dit proefschrift.

PUBLICATIONS

Refereed Scientific Journals

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Wientjes JGM, Soede NM, van den Brand H, Kemp B, 2010: Piglet uniformity and survival: effects of pre-ovulatory sow nutrition. In: Proceedings of the 14th Annual Conference of the European Society for Domestic Animal Reproduction (ESDAR), Eger, Hungary, September 15-18, 2010, pp. 53.

Wientjes JGM, Soede NM, van den Brand H, Kemp B, 2010: Effect of dextrose and lactose during the weaning-to-estrus interval on embryo development and uniformity in sows. In: Proceedings of the 14th Annual Conference of the European Society for Domestic Animal Reproduction (ESDAR), Eger, Hungary, September 15-18, 2010, pp. 73.

Wientjes JGM, Soede NM, van der Peet-Schwering CMC, van den Brand H, Kemp B, 2011: Piglet uniformity and mortality in organic sows; effects of pre-mating sow nutrition. In: Proceedings of the 36th Animal Nutrition Research Forum, Heverlee, Belgium, April 19, 2011, pp. 49-50.

Wientjes JGM, Soede NM, van der Peet-Schwering CMC, van den Brand H, Kemp B, 2011: Piglet uniformity and survival in organic sows: effects of pre-mating nutrition. In: Book of abstracts of the International Oskar Kellner Symposium, Warnemünde, Germany, September 9-11, 2011, pp. 21.

Wientjes JGM, Soede NM, Knol EF, van den Brand H, Kemp B, 2012: Pre-mating conditions, related with sow metabolic state, affect piglet birth weight uniformity. In: Proceedings of the 37th Animal Nutrition Research Forum, Wageningen, The Netherlands, April 18, 2012, pp. 71-72.

Wientjes JGM, Soede NM, van der Peet-Schwering CMC, van den Brand H, Kemp B, 2012: Piglet uniformity and survival in organic sows: effects of pre-mating nutrition. In: Book of abstracts of the 4th European Symposium of Porcine Health Management (ESPHM), Bruges, Belgium, April 25-27, 2012, pp. 117.

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Wientjes JGM, Soede NM, Knol EF, van den Brand H, Kemp B, 2012: Effects of pre-mating conditions on subsequent piglet birth weight and uniformity. In: Proceedings of the 17th International Congress on Animal Reproduction (ICAR), Vancouver, Canada, July 29-August 2, 2012, pp. 571.

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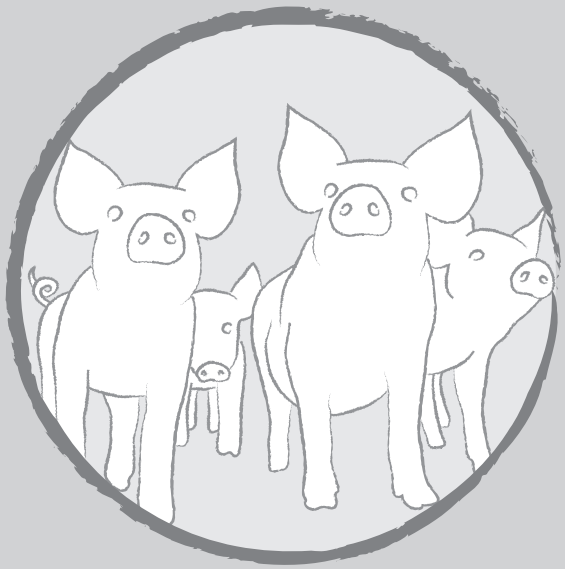
Other Publications

Wientjes JGM, Soede NM, van den Brand H, Kemp B, 2010: Effecten van eenvoudige koolhydraten en fermenteerbare koolhydraten op de binnen-toom variatie bij varkens. *Eindrapport voor het Productschap Diervoeder*.

WIAS Training and Supervision Plan	Year
The Basic Package (3.0 ECTS)	
WIAS Introduction course	2009
WGS course Ethics and philosophy in animal sciences	2010
International Conferences (5.4 ECTS)	
8th International Conference on Pig Reproduction (ICPR), Banff, Canada	2009
14th Annual Conference of the European Society for Domestic Animal Reproduction (ESDAR), Eger, Hungary	2010
Oskar Kellner Symposium – Metabolic Flexibility in Animal and Human Nutrition, Warnemünde, Germany	2011
4th European Symposium of Porcine Health Management (ESPHM), Bruges, Belgium	2012
17th International Congress on Animal Reproduction (ICAR), Vancouver, Canada	2012
63rd Annual Meeting of the European Federation of Animal Science (EAAP), Bratislava, Slovakia	2012
Seminars and Workshops (3.5 ECTS)	
WIAS seminar 'Aspects of lactation and weaning management for piglets and sows', Wageningen, The Netherlands	2009
'Denkaday' – Denkavit Symposium on animal nutrition, health and welfare, Voorthuizen, The Netherlands	2009
WUR symposium 'Pigs: The missing link?' Lelystad, The Netherlands	2010
WIAS seminar 'Animal Reproduction Research at ASG-WUR and FD-UU', Wageningen, The Netherlands	2010
WUR symposium 'Nutrition and sustainable pig production', Wageningen, The Netherlands	2011
WUR symposium 'Learning how to eat like a pig', Wageningen, The Netherlands	2011
WIAS Science Day	2010-2012
35th, 36th and 37th Animal Nutrition Research Forum, The Netherlands and Belgium	2010-2012
Presentations (8.0 ECTS)	
Oral presentation at ESDAR, Eger, Hungary	2010
Oral presentation at WIAS seminar 'Animal Reproduction Research at ASG-WUR and FD-UU', Wageningen, The Netherlands	2010
Poster presentation at WIAS Science Day, Wageningen, The Netherlands	2011
Oral presentation at Animal Nutrition Research Forum, Heverlee, Belgium	2011
Oral presentation at Oskar Kellner Symposium, Warnemünde, Germany	2011
Poster presentation at ESPHM, Bruges, Belgium	2012
Poster presentation at ICAR, Vancouver, Canada	2012
Oral presentation (invited speaker) at EAAP, Bratislava, Slovakia	2012

WIAS Training and Supervision Plan	Year
In Depth Studies (12.1 ECTS)	
Epigenesis and epigenetics, WIAS/VLAG, Wageningen, The Netherlands	2008
Vruchtbaarheid en voortplanting van het varken, WBS, Wageningen, The Netherlands	2009
Design of animal experiments, WIAS, Wageningen, The Netherlands	2009
Generalized linear models, PE&RC, Wageningen, The Netherlands	2009
Domestic Animal Reproduction – Ovary, NOVA, Helsinki, Finland	2010
Varkensvoeding in de praktijk, WBS, Wageningen, The Netherlands	2010
Carbohydrates with emphasis on nutrition and health of non-ruminant animals, Aarhus University, Tjele, Denmark	2011
Statistics for life sciences, WIAS, Wageningen, The Netherlands	2011
Professional Skills Support Courses (4.7 ECTS)	
Techniques for writing and presenting scientific papers, WGS, Wageningen, The Netherlands	2009
Information literacy PhD + Endnote introduction, WUR Library, Wageningen, The Netherlands	2010
Workshop Presentation skills, Language Services, Wageningen, The Netherlands	2010
Supervising MSc Thesis work, ESD&PS, Wageningen, The Netherlands	2010
PhD Competence assessment, WGS, Wageningen, The Netherlands	2010
PhD Career assessment, WGS, Wageningen, The Netherlands	2011
Workshop Stress identification and management, WGS, Wageningen, The Netherlands	2012
Research Skills Training (9.0 ECTS)	
Preparing own PhD research proposal	2008-2009
Talents & Topics program, WIAS, Wageningen, The Netherlands	2012
Didactic Skills Training (17.9 ECTS)	
Supervision yearly practical 'Reproduction and Fertility'	2009-2012
Supervision project group 'Inleiding dierwetenschappen'	2009
Supervision project group 'Sector integration course – Pig'	2011
Supervision project group 'Adaptatiefysiologie-2'	2011
Supervision practical 'Thermoregulation'	2011
Reviewing papers and judging research proposals of Research Master Cluster students	2012
Supervising 3 BSc students and 6 MSc students	2009-2012
Management Skills Training (6.0 ECTS)	
Member of WIAS Associated PhD Student Council	2009-2011
Organization of WIAS Science Day	2010 and 2011
Education and Training Total	**70 ECTS**

Dankwoord



De afgelopen vier jaar zijn voorbij gevlogen en met het verschijnen van dit proefschrift komt de eindstreep van mijn promotie-traject echt in zicht. Hiermee komt een einde aan een intensieve, maar bovenal ontzettend leerzame en leuke periode. Zowel het onderwerp en de praktische relevantie van dit onderzoek als ook de afwisseling in werkzaamheden (van lekker tussen de zeugen in de stal tot aan bloedmonsters analyseren in het lab en het uitpluizen van grote datasets) hebben hier zeker aan bijgedragen. Maar bovenal zijn het contact en de fijne samenwerking met een grote groep mensen hiervoor belangrijk geweest. Hartelijk dank allemaal! Een aantal personen wil ik in het bijzonder bedanken.

Allereerst mijn begeleiders: Nicoline Soede, Henry van den Brand en Bas Kemp. Nicoline, ik waardeer je enorme betrokkenheid, je directheid en je goede neus voor details en heb heel veel van je geleerd. Fijn dat je altijd even 'twee minuutjes' tijd voor me had! Henry, je bent altijd kritisch en durft altijd en overal je eigen mening te geven. Hiermee weet je vele anderen te prikkelen/inspireren en ben je een groot voorbeeld voor me. Bas, met je goede overzicht en creativiteit, en je altijd positieve kijk op tegenvallende resultaten, wist je me altijd weer te motiveren. Jullie enthousiasme, vertrouwen, inzet, positief kritische houding en het feit dat ik altijd bij jullie binnen kon lopen waren ontzettend waardevol. Fijn ook dat jullie als begeleiders op tijd mijn grenzen wisten bij te stellen als ik de lat voor mezelf weer te hoog had gelegd.

Dit proefschrift en de beschreven fysiologische proeven zouden niet mogelijk zijn geweest zonder de financiële steun van het Productschap Diervoeder. Daarnaast wil ik de leden van de werkgroep VWV bedanken voor de waardevolle discussies over proefopzetten, voersamenstellingen en resultaten. Ook wil ik Hennie Korten en Erik Franken bedanken voor het leveren van de zeugen, de bijkomstige werkzaamheden en de fijne samenwerking. Naast de grote fysiologische proeven, heeft de analyse van reeds beschikbare datasets mijn proefschrift extra inhoud gegeven. De medewerkers van varkensproefbedrijf Raalte en Carola van der Peet-Schwering en Gisabeth Binnendijk van Wageningen UR Livestock Research, bedankt voor het uitvoeren en beschikbaar stellen van gegevens van de 'suikerproef' en de hulp bij het analyseren van de data. TOPIGS Research Center IPG, bedankt voor het beschikbaar stellen van gegevens, de hulp bij het analyseren van de data en de interessante discussies over de resultaten, in het bijzonder Egbert Knol. Tevens wil ik Tette van der Lende bedanken voor zijn aanstekelijke enthousiasme en de leuke en nuttige discussies over de resultaten van dit proefschrift. Dank ook aan alle anderen die hebben geholpen bij het analyseren van de data en de interpretatie van resultaten, in het bijzonder Lisette Graat en Joost van den Borne. Dit hielp me steeds weer een stap verder!

Aan de voorbereiding en uitvoering van de fysiologische proeven hebben velen een steentje bijgedragen. Allereerst waren daar Bjorge, Rudie en Wouter. Bjorge, wat moet een 'repro-AIO' zonder jou beginnen? Jij zorgde ervoor dat de voorbereiding en uitvoering van de proeven soepel en volgens planning verliep en wist altijd de kalmte te bewaren. Bedankt voor al deze hulp, maar zeker ook voor alle gezelligheid onder werktijd en tijdens het carpoolen. Fijn dat je vandaag mijn paranimf wilt zijn! Rudie, bedankt voor alle hulp en gezelligheid in de stal en op Zodiac, maar bovenal voor de (hulp met) analyses van de vele bloedmonsters. Wouter, bedankt voor alle advies en de hulp bij de voorbereiding en uitvoering van de proeven in de stal en de analyses in het lab. Daarnaast wil ik alle andere collega's en alle studenten, in het bijzonder Dirkjan, Anniek, Els, Judith, Joost, Rianne, Fleur, Koen, Laura, Samantha en Xanthoula, bedanken voor hun hulp en gezelligheid in de stal en in het lab. Speciale dank ook aan de mannen van 'De Haar', in het bijzonder Ries, Ben en Rinie, voor het verzorgen van de zeugen en de assistentie in de stal.

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